

# THE BOTANICAL GAZETTE

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EDITOR  
EZRA JACOB KRAUS

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VOLUME 96

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WITH TEN PLATES AND FOUR HUNDRED AND EIGHTY-ONE FIGURES



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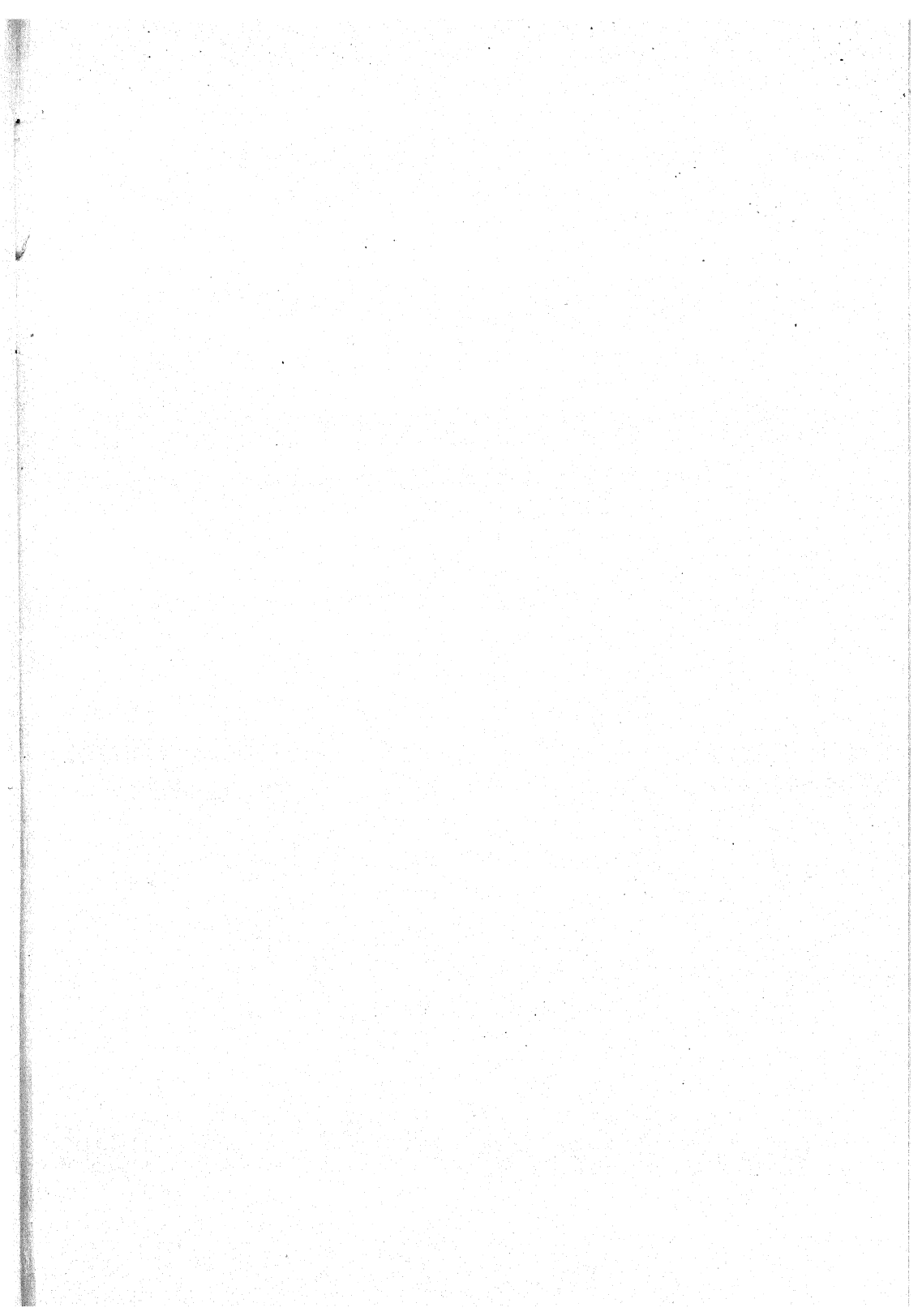
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## ERRATA

## VOL. XCV

P. 696, line 13 from bottom, for "3-5.5  $\mu$ " read "3-3.5  $\mu$ "





### HENRY CHANDLER COWLES

It is with keen regret that the editorial board of the BOTANICAL GAZETTE finds itself faced with the resignation of Dr. HENRY CHANDLER COWLES as editor. For the past nine years the journal has been under his leadership. He will continue as an associate editor of the GAZETTE, and impart to it his critical and careful guidance as he has done during the past years. Dr. COWLES is also retiring as Chairman of the Department of Botany, and has become Professor Emeritus.





# THE BOTANICAL GAZETTE

*September 1934*

POSTGLACIAL MIGRATION OF FORESTS IN ILLINOIS,  
WISCONSIN, AND MINNESOTA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 454

JOHN VOSS

(WITH FIFTEEN FIGURES)

## Introduction

The migration of biota initiated by the retreat of the Pleistocene ice sheets has long attracted the attention of investigators, especially ADAMS (3, 4, 5), GLEASON (19), HARSHBERGER (20), BRAUN (7), and TRANSEAU (35). The Pleistocene in North America lasted approximately one million years, in which time the northern half of the continent was glaciated during four distinct periods. An interval of deglaciation occurred between each period, long enough for the return and establishment of life on the newly uncovered land. During the last two decades several papers have appeared dealing with the biota of the interglacial periods, together with its significance in regard to the probable climatic conditions of those stages. Our knowledge of the types of vegetation and of the climate which existed during the interglacial epochs, especially the older ones, has been based mostly on the study of the macroscopic remains of plants and animals which lived during those periods. Deductions have also been made regarding climate and migration routes of the late interglacial periods from studies of the present distribution of biota, especially those of the relict type such as the boreal and the prairie (35, 43, 7).

LAGERHEIM (23), followed by VON POST (36) and others, introduced

the pollen analysis method as a means of attempting to solve the problem of interglacial changes. Since the introduction of this method very much work has been done in Europe, and in North America investigation is progressing rapidly, as shown by a recent map and bibliography published by ERDTMAN (16).

Through the study of the fossil pollen found at successive levels in peat deposits, the probable forest history of the area under consideration during the development of the bog may be reconstructed. Many factors must be taken into consideration before one can justly draw conclusions relative to biotic migration and climatic conditions. ERDTMAN (13, 14), STARK (34), AARIO (1), SEARS (30), and VOSS (38) have called the attention of workers in this field to the various sources of error.

One of the outstanding factors to be considered in the interpretation of pollen diagrams is that of pollen preservation. The conditions of hydration and acidity during the stages of development vary greatly. ERDTMAN (14) states: "Most of the pollen grains reaching the surface of a bog will not be preserved in a fossil state. The great supply of oxygen, the repeated wetting and drying, etc., which affect the pollen grains will hasten their destruction. The fossil pollen of the bogs, therefore, consists of those grains which were quickly carried down from the bog surface on which they landed to deeper levels with a smaller supply of oxygen and better conditions of preservation." In his work on the Canadian bogs (15) he again presents data showing that the height of the water table is an important factor in pollen preservation.

Account must also be taken of the variation in the quantity of pollen production by various genera found in the vicinity of bogs. Thus STARK (34) has shown that the amount produced by *Pinus* generally exceeds that of *Picea*. Likewise the quantity of pollen liberated by *Quercus* is less than that of *Fagus*. ERDTMAN (15) and AARIO (1), as a result of comparative studies of present forest composition and recent fossil pollen, emphasize the fact that the pollen spectrum is not always an exact picture of the forest in question.

A great variation exists in the resistance of various pollens to decay, even under conditions ideal for their preservation (29, 15, 27).

The question as to how far pollen travels is one that has been

considered by several workers. HESSELMAN (21) placed plates containing wet filter paper on ships in the Gulf of Bothnia and caught over 56,000 pollen grains of the genera *Pinus*, *Picea*, and *Betula* which had traveled more than 5.5 miles. JESSEN and RASMUSSEN (22) obtained in a treeless area on the Faroe Islands pollens of *Pinus*, *Alnus*, *Betula*, *Tilia*, and *Corylus* which had been carried more than 400 kilometers from their source.

In spite of its limitations, the record left by pollen is probably the most accurate one at present available, and in general, gives a rather satisfactory picture of the forests of the period.

### Geology

All the bogs under consideration are located within the limits of Late Wisconsin glaciation. At the beginning of substage III (LEV-ERETT), the center of ice movement appears to have been in the neighborhood of Patricia in Ontario. The glacier moved southward across Lake Superior and extended west as far as central Minnesota. It also covered most of the territory north of the driftless area and reached its maximum southern extension in northeastern Illinois and northwestern Indiana. The most pronounced moraine established in northeastern Illinois during this period is the one which parallels the present shore of Lake Michigan, averages about 10 miles in width, and is known as the Valparaiso moraine. LEIGHTON (24) states that at the close of the Late Wisconsin sub-epoch the ice melted rapidly, causing large blocks of ice to become detached and imbedded in the Valparaiso moraine. Upon the melting of these blocks, the small lake basins in that section of the state were formed. All the Illinois bogs described in this paper developed in lakes which were apparently initiated in this way. During the recession of the Late Wisconsin ice, the waters from the melting ice cut numerous channels which later served as excellent pathways for the advancing plants and animals. Regarding the interval between the receding ice and the appearance of plants and animals, LEIGHTON (24) states: "Judging from the freshness of contour of the glacial moraines, from the meager amount of gulying which has taken place, except under special conditions, and from the small amount of slope wash which has gone on, it appears that the increasing warmth responsible for

the melting of the ice made possible also a rather prompt reinvasion of the plant kingdom followed no doubt also rather promptly by a return migration of the animal life."

LEVERETT's substage IV was brought about by the shifting of the center of radiation from Patricia to Keewatin in central Canada. The ice moved southward through western Minnesota, eastern North and South Dakota, and south to Des Moines, Iowa. A minor lobe crossed the Mesabi range and another extended northeastward above St. Paul to Grantsburg and Osceola, Wisconsin. At approximately the same time, the Lake Superior lobe was reaching its maximum extension when it invaded the territory north of the lake, where it probably fused with the ice from central Canada. It also invaded the land southwest of Lake Superior, northern Wisconsin and Michigan, and eastern Wisconsin as far south as Milwaukee.

During the last sub-epoch of the Wisconsin, or LEVERETT's substage V, the ice moved from central Canada covering northern Minnesota, eastern North Dakota, northeastern South Dakota, and western Minnesota.

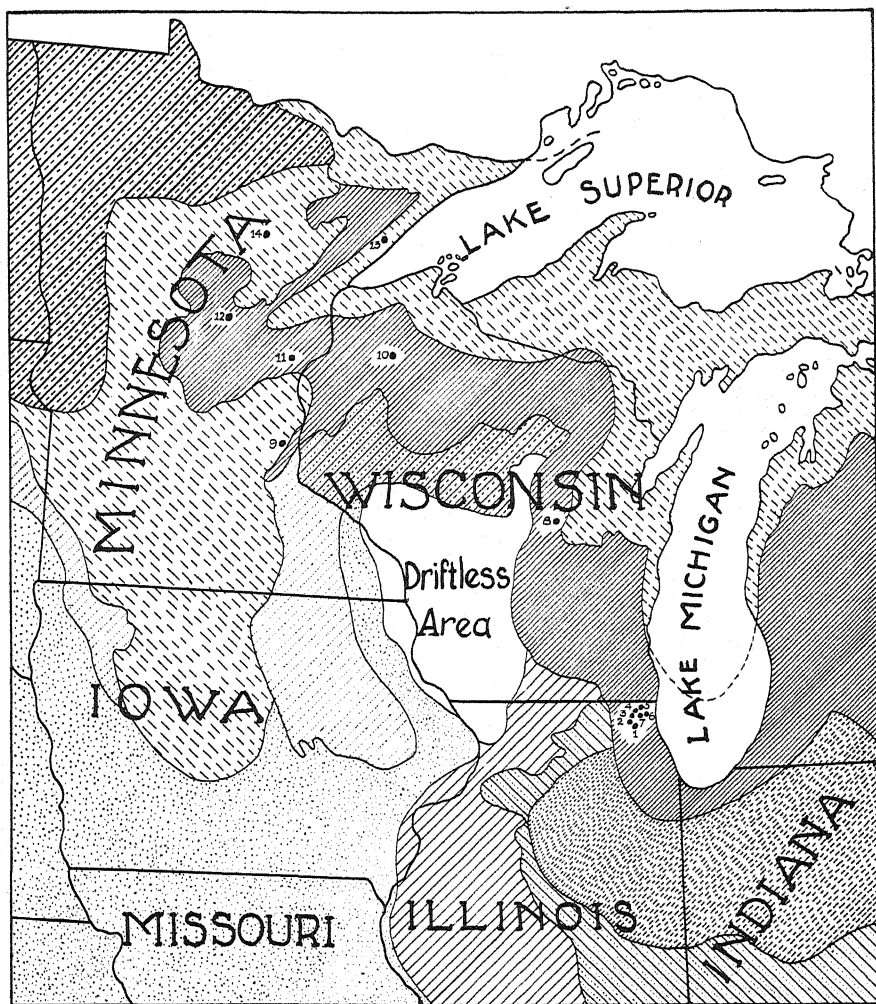
#### Location of bogs

Pollen analyses were made of fourteen bogs whose location with respect to the Wisconsin drift sheets is shown in figure 1. Cedar Lake, Millburn, Wauconda, Antioch, and Volo bogs have been well described by WATERMAN (39, 40).

Cedar Lake bog is very immature and occupies the northwestern part of Cedar Lake, in Section 32, Township 46 N, Range 10 E, Lake County, Illinois. Near the edge of the open water the surface of the mat is quaking and the peat is over 40 feet deep. In order to reach the bottom of the lake, borings were made in the swamp zone about 100 feet from the edge of the mat.

Millburn bog is approximately 500 yards long and 300 yards wide, and is found in Section 35, Township 46 N, Range 10 E, 1 mile west of the village of Millburn, in Lake County, Illinois. The substratum is firm and covered with sphagnum, pitcher plant, and cranberry.

The bog at Wauconda is located southeast of the city of Wauconda in Section 25, Township 14 N, Range 9 E. The surface of the bog is solid with its northern half covered with tamarack and the remainder with grasses.



(AFTER LEVERETT, LEIGHTON, MARTIN.)

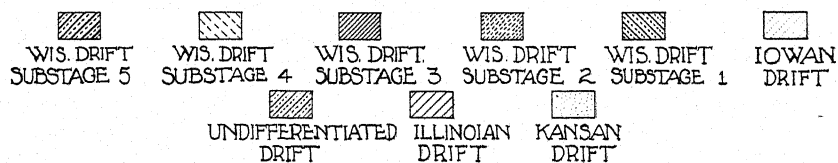


FIG. 1

The Antioch bog occupies a circular depression 300 yards in diameter, in Section 15, Township 46 N, Range 10 E, 3 miles southeast of Antioch, Lake County, Illinois. It is mature and bears several old trees of tamarack and yellow birch.

The Volo bog occupies an oval depression about 0.75 mile long and 0.5 mile wide, and is found on the George Sayer Farm no. 3 in Section 28, Township 45 N, Range 9 E. Open water is found in the center, surrounded by a quaking mat 75 yards wide.

The Gavin bog, so named by the writer on account of its proximity to the Gavin school, is a peat-filled lake extending north and south in Section 12, Grant Township, Lake County, Illinois. The substratum is very solid and the southern portion bears many mature tamarack trees.

The Hastings bog extends north and south in sections 22 and 27, Antioch Township, Lake County, Illinois, and is approximately 900 yards long and 200 yards wide. A small pond is found near the center and all borings were made near the north margin of the pond. Practically all the vegetation on the bog was destroyed by fire shortly before the writer visited it.

The Waupaca bog covers an area of about 60 acres and is located about 2.5 miles north of Waupaca, Wisconsin, along the tracks of the Waupaca & Green Bay Railroad. The substratum is quaking and the cover consists chiefly of sphagnum, leatherleaf, bog rosemary, Labrador tea, tamarack, and black spruce.

The Bald Eagle bog is about 0.5 mile east of Bald Eagle, Minnesota, near the boundary of Ramsey and Washington counties, in Section 1, Township 30 N, Range 22 W. It is a peat-filled lake with a non-quaking surface covered with sphagnum, alder, a few black spruce, and many large tamaracks.

The Hayward bog is located 3 miles west of the city of Hayward, Sawyer County, Wisconsin, just north of the main road running west of the city. It is bordered on the north by a low ridge, covers an area of about 200 acres, and represents a filled lake with sand forming the bottom. A narrow private road traverses the bog in a north-south direction. The bog is mature and the principal vegetation consists of tamarack, black spruce, sphagnum, pitcher plant, cranberry, Labrador tea, bog rosemary, and cassandra.

Mission Creek bog comprises an area of about 1 square mile and is located 1 mile south of Mission Creek station, along the Northern Pacific Railroad in Pine County, Minnesota. It represents a peat-filled lake. The area has been partially drained and originally was a tamarack swamp, as evidenced by the few remaining dead trees and the numerous stumps in the upper 4 feet of peat. The upper surface of the peat is firm and mostly covered with herbaceous vegetation.

Bay Lake bog is found just south of Bay Lake in Section 27, Bay Lake Township, Crow Wing County, Minnesota. It also is a peat-filled lake, having a sand and clay bottom. It is elliptical in shape, approximately 1 mile long and 0.25 mile wide, and is covered principally with sphagnum, heath shrubs, and scattered tamarack and black spruce.

The Highland bog occupies a depression between two low ridges 1 mile north of Highland, Lake County, Minnesota, along the Duluth & Iron Range Railroad. It is about 80 acres in area and consists of a filled lake, the surface of the deposit having been raised above the old water level by successive layers of sedges and sphagnum. The bog is covered with a dense growth of sphagnum, young tamarack, and black spruce.

The Coleraine bog is a small filled lake covering an area of about 12 acres, located 1 mile west of Coleraine, Itasca County, Minnesota, along the Great Northern Railroad. On the north side a steep narrow ridge separates the bog from a small lake. The bog is mature, covered mostly with sphagnum, and has a few small tamarack and black spruce trees at the west end.

### Methods

The samples of peat used for pollen analysis were obtained by means of a Hiller peat augur, the construction and operation of which have been described previously (37). Borings were made along a line across the longest portion of the bogs and the drillings were generally 100 feet apart. The method as described by Voss (37) was followed in the preparation of the material for identification and counting. The identification of the pollen was carried out by means of type slides of fresh pollen and the use of SEAR's drawings (30).



At least 150 pollen grains were counted and identified for each sample, and the pollen frequency, or number of pollen grains per square centimeter, determined. According to ERDTMAN (12) reliable percentages are obtained if 150 pollen grains are counted, although BOWMAN (6) has expressed a contrary opinion.

### Results

Figures 2 to 15 show the percentages of fossil pollen and the pollen diagrams of the dominant trees, the minor ones having been omitted from the diagrams to minimize complexity. The numbers at the left of the diagrams represent the depth of the peat in feet or meters and the numbers in the horizontal scale give the percentage of tree pollen. The trees are indicated by signs, which are indexed. The stratigraphy is shown by a diagrammatic section. The type of peat designated as limnic consists of all material deposited under water. At the right of each diagram the pollen frequency curve is shown.

In all the Illinois bogs under discussion, *Abies* and *Picea* pollens reached their maximum at the bottom of the bogs and decreased nearer the surface, the decrease in some cases being gradual while in others it was abrupt. *Quercus* pollen was found in all the bottom peat and its percentage gradually increased toward the surface, the percentage remaining rather constant during the development of the major portion of the bogs. *Pinus* pollen was found at practically all levels and its percentage was also rather constant in all bogs from bottom to top. *Larix laricina* wood was encountered at the bottom of Cedar Lake, Gavin, and Millburn bogs. This wood was probably imbedded in the blocks of ice which were responsible for the original formation of the lake.

In the Waupaca (fig. 9) and the Hayward (fig. 11) bogs also, *Abies* was the dominant tree at the bottom, constituting 42 and 59 per cent respectively of the entire pollen. In both bogs *Picea* pollen was most abundant at the lowest levels, decreased toward the center, and increased nearer the surface. The *Pinus* curve of the Waupaca bog culminated at the 19 foot level and in the Hayward bog at the 8 foot level. The increase near the surface may be due to the presence

of the bog spruce, *P. mariana*, on the surrounding mature bogs. The percentage of *Quercus* pollen in the Waupaca bog was much higher than that of the Hayward bog; with an increase of *Quercus*, there was generally a decrease of *Pinus* pollen.

The pollen frequencies of the peat between the 5 and the 15 foot levels of the Mission Creek bog (fig. 12) were so low that the counts for those levels were omitted from the table and diagram. The low frequencies are perhaps due to the manner in which the peat was built up above the water level. DAVIS (10) emphasizes the fact that when the peat is built up above the water level, new species and more individuals appear, resulting in much dead material coming in contact with the air and the heat of the sun. This causes great loss of water and permits the growth of organisms which promote decay, thus destroying the pollen grains. The greater pollen frequency in the upper 5 feet was probably due to a change in the water content of the peat. *Abies* was the most significant tree nearest the bottom and *Pinus* and *Picea* at the top, with *Quercus* playing only a minor rôle throughout.

The pollen diagram of Bay Lake (fig. 13) is in many respects similar to that of Waupaca, the chief difference being the greater abundance of *Pinus* and *Picea* pollens and less *Quercus* pollen.

Three of the Minnesota bogs, Bald Eagle, Coleraine, and Highland, are located within substage IV of the Wisconsin epoch, and, according to LEVERETT (personal communication), are probably several thousand years younger than the other bogs in Minnesota, Wisconsin, and Illinois. WILSON (42) has shown that an interval existed between substages III and IV long enough for the development of a forest in eastern Wisconsin in which trees at least 80 years old were found.

Very low pollen frequencies were also obtained from the analysis of the upper 13 feet of the Bald Eagle peat, the building up process probably having taken place under conditions like those of Mission Creek.

## PERCENTAGES OF FOSSIL POLLEN: WAUCONDA BOG

Depth Ft.	Abies	Larix	Picea	Pinus	Acers	Alnus	Betula	Carya	Celtis	Carpinus	Ostrya	Fraxinus	Juglans	Populus	Quercus	Salix	Tilia	Ulmus
3				13.3			3.4	3.4				.8	3.4		75.5	.8		.8
4				17.6	1.4		5.8	5.8	1.4				1.4		58.7	2.9		4.4
5				11.8	3.2	.6	3.2	9.2	1.3	1.9	1.3		.6		61.8	1.3	1.3	1.9
6		2.7		13.6			2.0	6.1					.6	.6	71.2			.6
7				19.5			1.3	2.6							76.0			.6
8				17.7				2.7							78.0			1.3
9				11.0		.6	1.3	3.2							82.0			1.9
10				13.9			.6	3.8					.6		78.0			3.1
11				13.8				4.6							81.0			.6
12				15.0			.6	6.5							74.5	1.3	1.9	
13				12.6			1.9	4.6							77.0	.6	3.3	
14				13.9		.6	1.3	7.3							76.0			.6
15				14.8			.6	6.4					1.2		70.5	1.2		5.1
16				8.9			1.2	9.5							71.0	.6		8.9
17				6.5			.6	5.9							76.1	1.3	9.2	
18				12.2			.6	3.8							79.0	.6		3.2
19	1.2		3.1	21.3			3.1	5.6							46.0	.6	5.0	13.8
20	.6		4.2	18.2			6.0	1.8							53.2	1.2	.6	13.9
21	24.1		8.0	20.7			3.4	4.6							34.4	1.1	3.4	

FIG. 2A

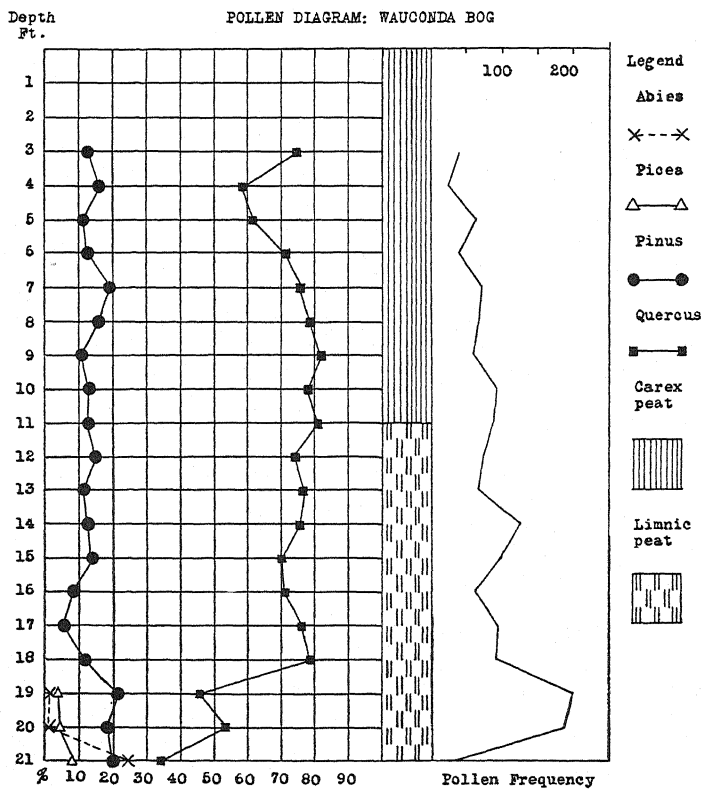


FIG. 2B

## PERCENTAGES OF FOSSIL POLLEN

## VOLO BOG

Depth Ft.	Abies	Picea	Pinus	Tsuga	Alnus	Betula	Carya	Celtis	Carpinus	Ostrya	Fraxinus	Juglans	Populus	Quercus	Salix	Tilia	Ulmus
1			26.0		3.4	.6	1.3	2.0					.6	60.0	.6		
2			12.2		1.9	1.2	3.2	3.8	1.2	.6	.6	.6	.6	66.3	1.2	2.8	3.2
3			12.9	1.9			5.8	2.5	.6		.6		1.2	66.7	1.8		.6
4			13.2	.6	.6	.6	4.2	6.0		.6	.6			72.2			.6
5			11.3	.6			2.9	7.7	2.9				1.7	71.0			1.7
6			9.8	.6			2.6	7.2	.6			.6	2.6	72.5		1.3	1.9
7			5.6		1.2		1.8	8.7	1.2			1.2		77.2			2.5
8			10.9				3.0	6.6	1.2					77.0		1.2	
9			9.3				3.7	8.1				1.2		73.9		.6	3.1
10			6.0				.6	8.7	1.3					80.5	.6	.6	1.3
11			14.0					6.6						76.5			2.6
12			13.2				.6	5.9	1.3			2.6		75.8			.6
13			6.3				1.9	5.0	.6			1.2		83.0			1.9
14			5.2	.6			2.6	5.2						85.5		.6	
15			6.1				1.2	7.2				.6	.6	80.1			4.2
16			8.3				1.2	5.7						81.5		.6	2.5
17			11.3					5.6				.6		78.5		.6	3.1
18			10.6			.6		5.6				1.2		79.2		.6	2.5
19			12.1				1.2	4.4				.6		78.2		.6	2.5
20			6.8				.6	4.9				.6		78.8		.6	8.0
21			6.8				3.1	5.5						79.4		1.2	3.7
22			7.6				4.5	8.3		.6				73.7		1.9	3.2
23			7.5				.6	6.2						82.3		.6	2.5
24			15.4				1.2	5.1						72.8			5.1
25			21.4				5.8	5.1						64.6		.6	1.9
26			9.5				1.3	1.3	.6					85.5		.6	
27			25.6				6.5	3.5						61.0			4.1
28			22.7				3.7	2.5	.6			.6		53.2		1.2	14.5
29	25.2	19.5	21.4				11.2	3.9						15.6			3.2
30	47.7	35.0	10.7				2.1	.7						2.1		.7	.7
31	56.5	16.7	15.4				4.4							5.1			2.9
32	69.4	14.9	4.8				3.2							7.1			1.3
33	81.0	4.3	1.8				5.5							6.2			1.2
34	79.5	3.8	3.1				3.1	.6						7.6			1.9
35	90.5	5.1	1.2				.6							1.9			.6
36	86.4	3.0					4.5							3.0			3.0

FIG. 3A

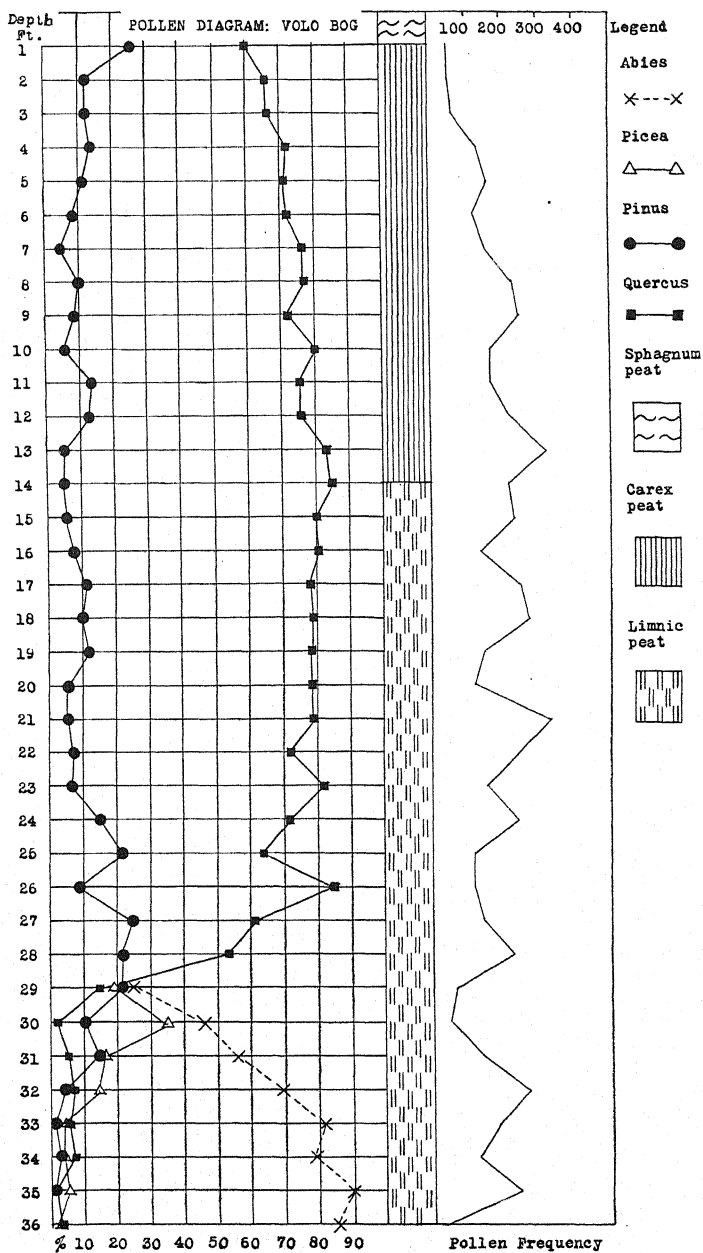


FIG. 3B

PERCENTAGES OF FOSSIL POLLEN: GAVIN BOG

Depth Ft.	Abies	Picea	Pinus	Alnus	Betula	Carya	Fraxinus	Juglans	Populus	Quercus	Salix	Tilia	Ulmus
1			26.0	.6	4.0	4.6				64.0	.6		
2			7.2		3.9	4.6				83.0			1.3
3			16.5		2.6	3.9		.6		73.0	.6		2.6
4			19.3		5.1	1.9		.6		67.5	3.2		1.9
5			10.1	1.0	3.0	3.0		1.0		77.5	3.0		1.0
6		.6	14.4	.6	3.2	7.2				69.0	1.9		2.6
7			8.1		2.5	8.1				80.0			1.2
8			11.7		1.3	7.8				78.0	.6		.6
9			16.0		3.3	7.7		.6	1.3	70.0		.6	.6
10			7.0		1.2	5.7	1.2	.6		76.0	1.2	2.5	3.2
11			9.4		.6	6.9	1.8	3.1		73.5		1.8	2.5
12		1.3	5.9		1.3	5.2		1.3	.6	79.5		.6	3.9
13		.6	7.8		2.6	1.9		1.9		79.0		1.9	3.9
14			5.2		.6	3.9		.6	1.3	78.0		3.2	6.6
15		1.2	3.8		1.9	1.9				82.0		2.5	7.0
16		2.8	7.3		3.4	3.9	1.1	1.1	1.1	68.5	1.1	3.4	6.2
17	.6	4.3	8.6		1.8	.6				73.5	1.2	4.9	4.3
18	1.7	2.8	13.8		4.0	1.1				54.8	1.7	8.1	11.6
19	14.6	13.4	7.6		7.6	.6				47.9	1.2		7.0
20	7.9	8.5	14.7		3.0					54.5	2.4	4.9	3.6
21	37.5	13.2	10.0		9.4	1.8				20.6	1.8	.6	5.0

FIG. 44

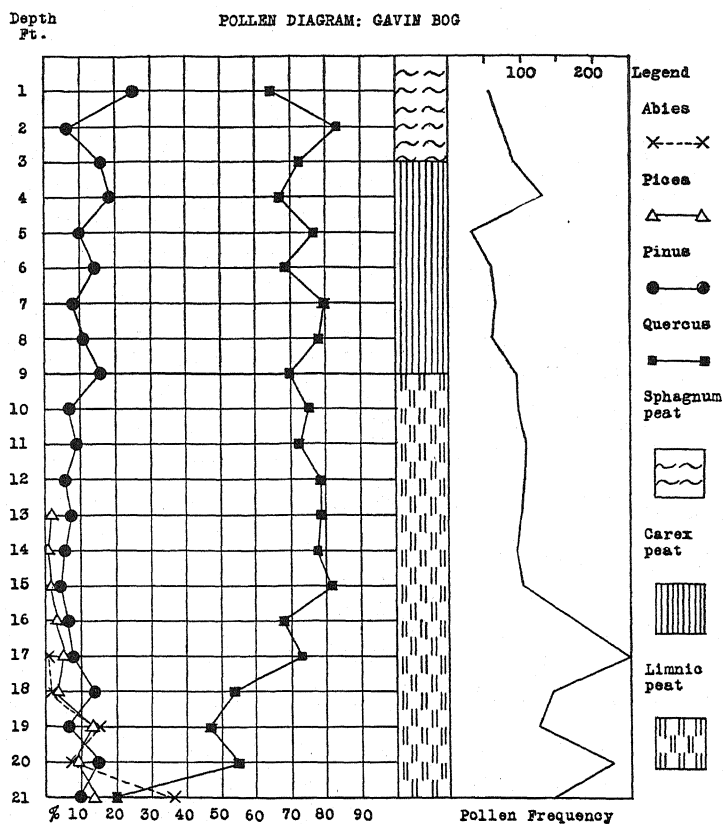


FIG. 4B



## PERCENTAGES OF FOSSIL POLLEN

## CEDAR LAKE BOG

Depth M.	Abies	Picea	Pinus	Tsuga	Acer	Alnus	Betula	Carya	Carpinus	Celtis	Juglans	Populus	Quercus	Salix	Tilia	Ulmus
.50			1.2		2.1		2.1						81.0			
.78			32.2				7.5	1.8					57.0		2.2	
1.00			23.5				2.8	1.0					71.5			.9
1.22			17.4				2.0	1.3					78.0			1.3
1.50			15.8				4.7	3.9					75.0			
1.72			13.1				2.9	2.6					78.0		.6	1.9
2.00			11.4				2.6	2.0			1.3		81.0			1.3
2.22			11.9	.6			2.6	.6			.6		82.5		.6	
2.50			16.4	.6			3.8	2.5			1.9		75.0			
2.72			14.4	.6			3.9	1.3	.6		1.3		75.5		1.3	1.3
3.00			6.6				2.6	1.9					88.0			.6
3.22			9.8				1.3	2.6	.6				82.0		1.9	1.9
3.50			10.6				2.0	2.0					82.0		1.3	2.0
3.72			11.0	.6			1.3	2.6					82.5		.6	1.3
4.00			15.6	.6	.6		1.9	2.6					77.0		.6	.6
4.22			17.1				2.5	1.9					76.0		.6	1.2
4.50			28.7	.6			1.9	2.5	1.2				61.2		1.2	1.9
4.72			23.6	.6	1.2		1.9	3.8	.6		.6		64.0		1.2	1.9
5.00			14.0		1.9		1.3	5.9	.6				76.0			
5.22			11.8		3.4		8.4	3.4					71.0			1.7
5.50			18.7				3.4	3.4					73.0			.6
5.72			20.0				3.3	2.0					74.0		.6	
6.00			15.0				3.2	3.9					76.0		.6	1.3
6.22			13.0				4.5	1.3					78.0		1.9	.6
6.50			9.0				.6	3.8					85.0		.6	.6
6.72			7.2				2.6	3.9		1.3			82.0		1.3	1.3
7.00			20.5				2.6	5.3	.6				68.7		1.3	.6
7.22			24.5	.6			1.3	1.9		.6			68.0		1.3	1.3
7.50			15.0				1.3	3.2					76.5		.6	3.2
7.72			11.3	.7			1.4	2.8					81.5		.7	1.4
8.00			14.6				.6	1.3	1.9		.6		79.0		1.9	
8.22		.6	6.6				1.3	2.6	2.6				80.0		.6	5.3
8.50			12.2				.6	5.1	2.2				75.0		1.2	2.5
8.72		.6	13.0				1.8	2.4	1.8				75.0	1.9	1.2	1.8
9.00		.6	16.8				1.3	3.3	1.3				66.5	2.0	1.3	6.7
9.22		2.6	20.8				1.9	1.9	1.3				60.0	.6	3.2	7.2
9.50	2.0	4.0	9.2				1.3	.6	1.3				63.5	.6	.6	.6
9.72		2.1	25.2				1.4	2.8	.7				63.0		2.1	2.8
10.00	20.2	20.9	28.3			.6	4.0	.6	.6				21.0			3.3
10.22	29.7	58.0	7.5				.7		.7				3.8			
10.50	27.0	61.0	3.2				.6						7.1			.6
10.72	50.0	34.5	5.4				2.7				.6		6.7			
11.00	64.0	14.1	2.5				3.8				.6		13.6			1.2

Fig. 5A

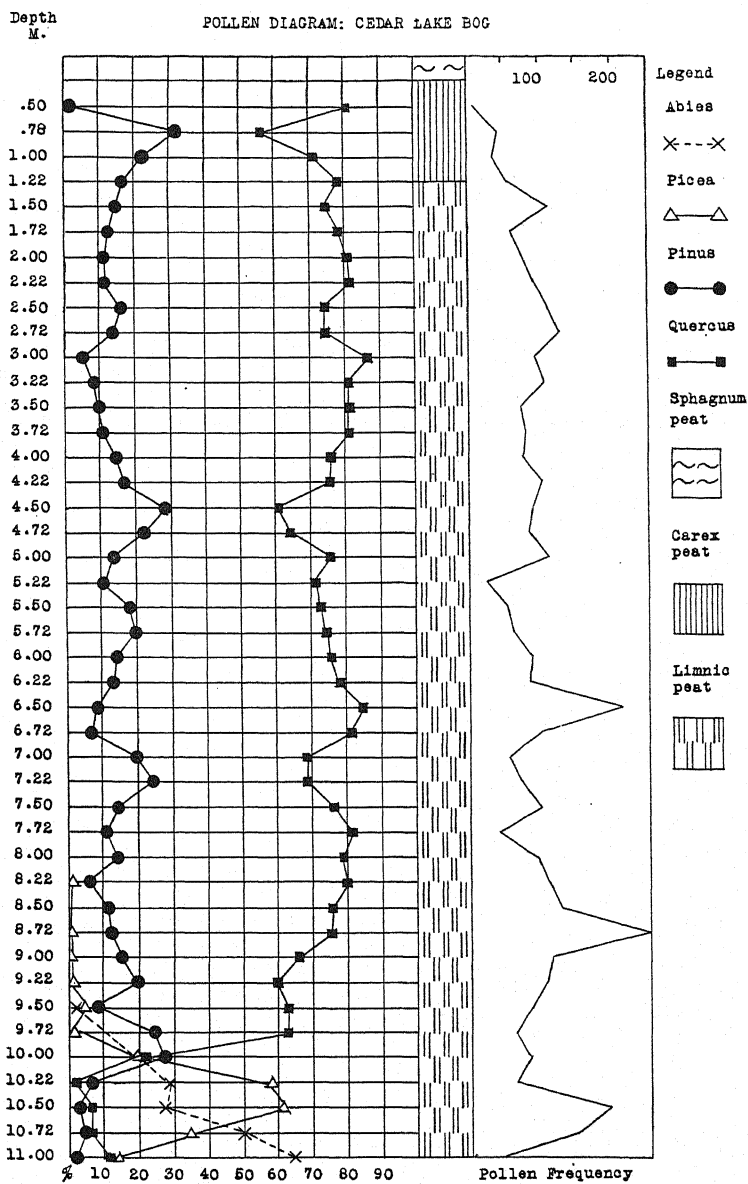


FIG. 5B

## PERCENTAGES OF FOSSIL POLLEN: ANTIOCH BOG

Depth Ft.	Abies	Picea	Pinus	Tsuga	Alnus	Betula	Carya	Celtis	Carpinus	Corylus	Ostrya	Juglans	Quercus	Tilia	Ulmus
2			7.9			4.6	4.6						82.0		.6
3			10.6			1.9	.6						84.0		2.6
4															
5			14.6			2.4	1.2						82.0		
6			10.5			2.9	1.4						84.0	1.4	
7			11.2			2.4	7.4	3.7					75.5		
8			7.2			1.3							88.0		3.3
9			8.1	.6		.6	6.1			1.3		.6	74.0	.6	7.5
10			12.2			1.9	1.9		.6	2.5			76.0		4.5
11			4.1			.6	2.0	.6		.6		1.3	82.0	.6	7.5
12			3.8				6.3					.6	80.0	.6	8.2
13			4.0				3.3						78.0		14.2
14			2.5			1.2	3.7	.6					78.0		14.4
15			3.7			1.2	3.1						78.0	.6	13.2
16			7.0			2.5	1.2			1.2			79.0	.6	7.7
17			6.0	.6		1.8	2.4			1.2		.6	75.0		9.9
18			8.2			.6	4.4					.6	81.0	.6	4.4
19			12.1				3.2						74.5	1.9	8.3
20		.6	8.7	.6		2.0	2.6			.6		1.3	68.0	2.0	12.7
21	1.3	1.3	16.4			3.2	1.3	1.3		1.3	2.6	1.3	47.0	1.3	21.5
22	30.5	18.4	9.5		.6	8.3	.6						17.2		14.6

FIG. 6A

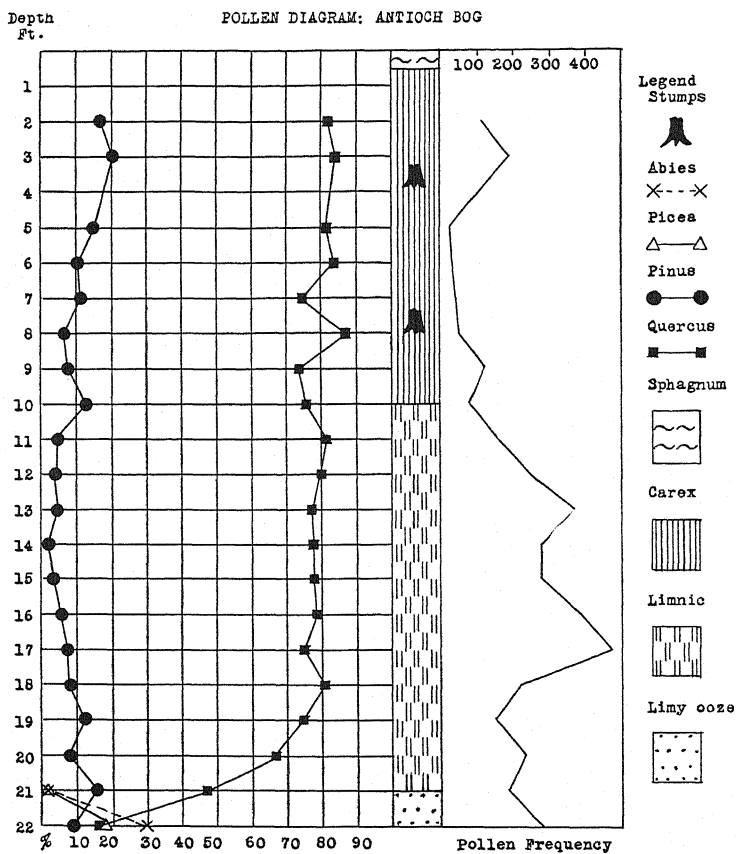


FIG. 6B

## PERCENTAGES OF FOSSIL POLLEN

## MILLBURN BOG

Depth M.	Abies	Picea	Pinus	Tsuga	Betula	Carya	Carpinus	Juglans	Quercus	Salix	Tilia	Ulmus
.35			13.6		11.0	5.2	.6		61.7	7.1		.6
.72			24.7		4.3	1.4			64.1	5.1		
1.00			18.0	.81	2.4	1.6		.8	71.5	3.2		1.6
1.72			6.4		3.2	2.5	.6		83.2	2.5		1.2
2.00			8.5	.6	.6	3.2	.6		80.3	5.2		.6
2.72			7.7	.6	3.8	4.5		.6	78.0	2.5		1.9
3.00			15.3	1.8	1.2	6.1	1.2		68.5	1.8	.6	1.2
3.72			11.7		3.1	9.2	1.2		69.8	1.2		3.7
4.00			12.4		3.9	6.5	1.3		74.6	.6		.6
4.72			14.2		2.5	3.2			77.2			2.5
5.00			5.8	1.2	2.5	3.8			81.0	1.9		3.2
5.72		.6	8.1		.6	3.4	.6		83.8	2.7		
6.00		.8	13.4		2.6	7.1			74.0		.8	.8
6.72			11.3		2.0	3.3			81.3		.6	1.3
7.00			11.6		1.9	3.9			78.0		.6	3.9
8.22			5.8		1.2	1.9			85.5	.6	.6	3.8
8.36			8.4		3.2	1.3	.6		78.4	.6	.6	6.5
8.50			19.4		.6	1.2	.6		71.0		3.1	3.1
9.47	2.8	5.4	20.3		8.1	1.1	.5		52.5		.5	8.6
9.75	17.2	54.9	14.6		6.3	.6			6.3			
10.47	35.6	18.7	6.6		4.6	4.0			22.0	2.6		4.6
10.61	74.8	18.7							6.6			

FIG. 7A

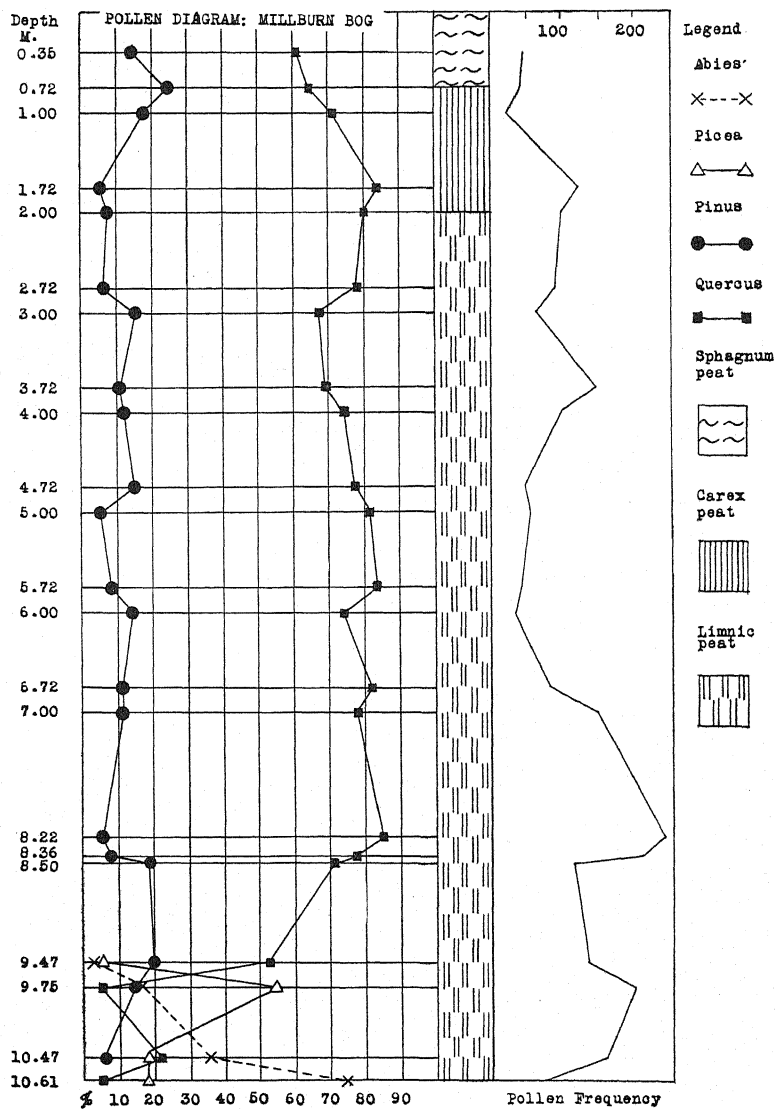


FIG. 7B

## PERCENTAGES OF FOSSIL POLLEN

## HASTINGS BOG

Depth Ft.	Abies	Picea	Pinus	Alnus	Betula	Coryla	Juglans	Populus	Quercus	Salix	Tilia	Ulmus
1			13.6		1.6	4.8	.8		75.0	2.4		1.6
2			11.3		.8	5.6	.8		68.0			
3			12.2		3.8	1.9			61.2			.6
4			5.9		1.4	4.4			88.0			
5			12.1		1.9	2.5			82.0			1.2
6			10.6		2.5	2.5			82.0	1.2		1.2
7			12.7		.7	3.7			61.2	.7	.7	
8			12.8	.9	1.9	3.8	1.9		77.0	.9		1.2
9			6.6			3.9	1.3		87.0		.6	
10			6.8		2.2	3.1	.7	.7	87.0	2.2		.7
11			16.8		2.6	1.7			78.0	.8		
12			13.7		3.9	2.9	.9		76.5	.9		.9
13			8.1		4.0	1.6			87.0	1.6	.8	
14			10.2		1.7	.8			66.5	.8		1.2
15			10.0		1.2	1.8			85.2			
16			11.4		2.0	1.3			84.5			.6
17			5.5		.7	3.1	.7		87.1	.7	.7	.7
18			8.2	.6	1.9	6.9	.6		79.5			1.9
19					.6	3.7			88.0		1.8	5.5
20			3.9		.6	2.6			89.2		1.3	1.9
21		.6	16.2		1.2	1.8			75.0	.6	1.8	2.5
22			13.5		3.2	3.2			75.0			4.5
23		5.1	15.9		3.8	3.1			54.0		5.7	12.1
24		2.4	17.5		3.6	4.2			56.2	.6	1.8	13.3
25		1.2	15.9		5.7	1.9			62.3	1.9		10.8
26	8.7	11.4	17.5		19.0	.6			24.6	1.9	1.9	13.9
27	39.0	13.7	20.2		9.1				13.7			4.5
28	72.1	13.2	5.9		1.9				5.9			.6
29	73.5	9.2			2.6				13.2			1.3

FIG. 8A

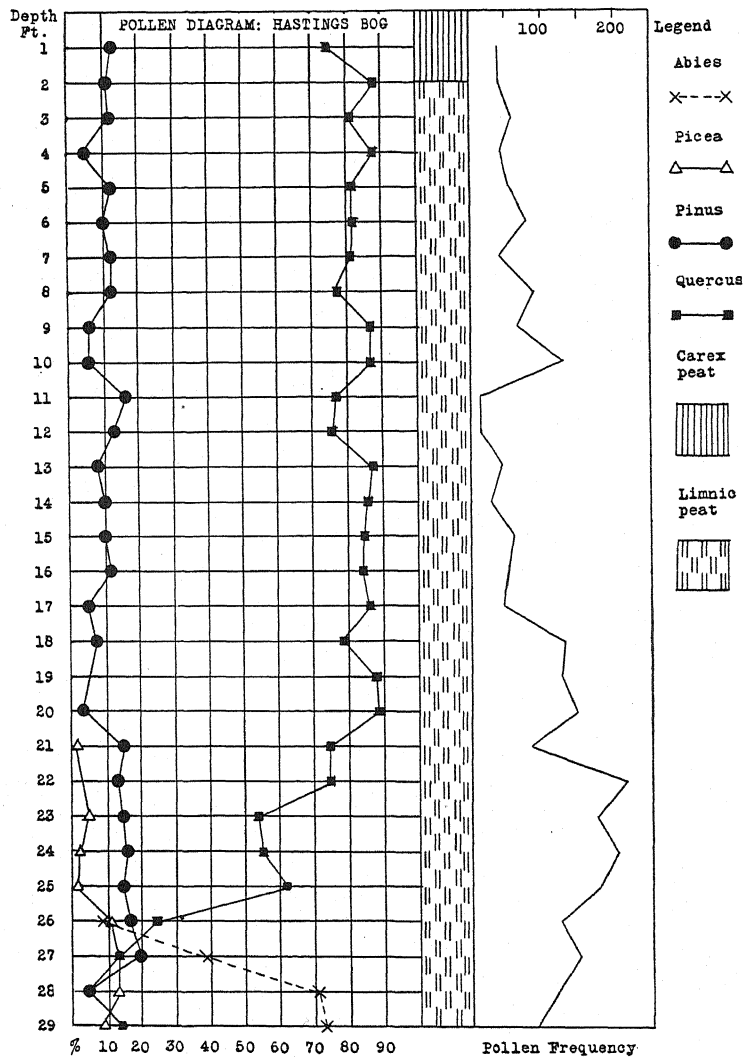


FIG. 8B



## PERCENTAGES OF FOSSIL POLLEN

## WAUPACA BOG

Depth Ft.	Abies	Picea	Pinus	Taxus	Alnus	Betula	Carya	Carpinus	Corylus	Ostrya	Fagus	Juglans	Quercus	Tilia	Ulmus
1		19.3	42.5	1.3	3.9	5.3		2.6	.6			1.3	21.9	.6	
2		17.8	41.5	4.7	3.5	7.1	.5	2.6		.6		1.1	19.6		1.1
3		11.0	33.5	4.0	5.8	4.6	1.1	2.3	.5	1.1		.6	34.6		.5
4		4.6	37.0	3.9	7.2	3.9	1.3	1.9		1.3		1.9	36.0		
5		6.0	37.0	2.4	6.0	3.0	.6	2.4			1.2	1.8	39.0	.6	
6		3.8	32.5	1.9	7.6	2.5		1.2			1.9	.6	48.0		
7		5.8	34.0	2.3	7.5	2.3		1.7			1.7	2.3	42.0		
8		3.2	26.2	1.2	6.7	3.2	.6	3.2			.6	2.6	53.0		
9		2.0	26.0		6.0	2.5	.6	2.5			.6		57.0		2.0
10		3.0	33.4		3.0	2.4	1.2	1.2	.6		1.2	1.2	52.5		
11		2.6	32.5		3.2	3.2		1.9	.6	.6	3.9	1.9	46.0	.6	2.6
12		1.7	38.7		4.7	1.1		2.3			2.3	.6	42.2	.6	5.3
13		1.2	49.0	1.2	2.4	1.2		.6			1.8		38.0		4.8
14		3.5	43.5		1.7	2.3	.5	.5		1.1	1.7		40.0		4.8
15		1.9	43.0		2.5	.6	.6				1.9	1.9	41.5	.6	5.1
16		3.7	53.0		3.1	1.8					2.5	.6	28.5	.6	6.2
17		2.0	52.8		5.4	2.7	.6	1.3			.6		20.3	.6	3.3
18		7.8	64.3		1.9	1.2	.6				1.2	.6	16.1		5.8
19		3.9	68.0		3.9	1.3	.6						17.0		5.2
20		1.7	66.0		5.9	1.1	1.7	1.1					18.2	.5	3.5
21	4.3	36.2	38.5	.6	2.5	3.1		1.2					11.8	.6	.6
22	32.5	45.8	12.1			1.2						.6	7.0	.6	
23	42.7	42.0	11.6			.6							2.6		

FIG. 94

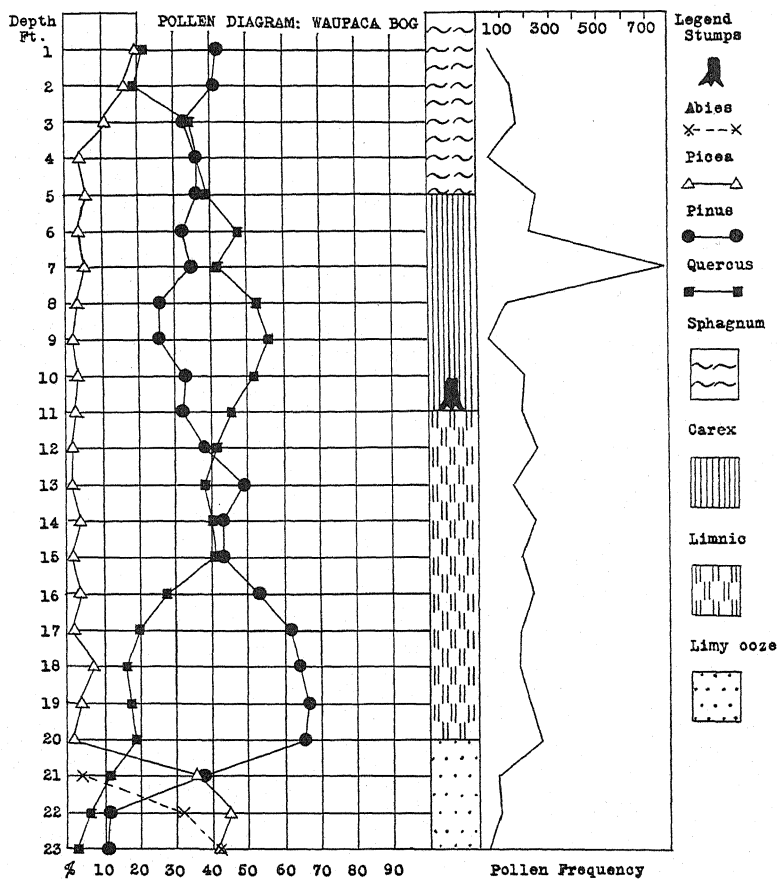


FIG. 9B

## PERCENTAGES OF FOSSIL POLLEN

## BALD EAGLE BOG

Depth Ft.	Abies	Picea	Pinus	Alnus	Betula	Corylus	Populus	Quercus	Salix	Tilia	Ulmus
13		12.4	49.3	.6	2.5			26.2	1.3		5.9
14	.6	12.9	51.5	.6	4.5	.6	1.9	15.4	1.9	3.8	5.8
15	1.8	22.0	51.2	3.5	2.4			9.7	.6	1.2	7.9
16	9.6	48.0	25.5		10.9			3.8			1.9
17	83.5	8.5	2.6		2.6			1.9			.6
18	79.5	15.7	3.9		.6						
19	81.0	15.0	1.9		1.3			.6			
20	87.5	9.4	.6		1.2						

FIG. 10A

## POLLEN DIAGRAM: HAYWARD BOG

Depth Ft.	Abies	Picea	Tsuga	Betula	Carya	Ostrya	Quercus	Salix	Tilia	Ulmus
1		25.5	62.5	7.5			4.3			
2		25.9	66.0	2.4			3.7	.6		1.2
3		10.2	76.0	2.5			6.9	3.1		1.2
4		3.6	77.0	3.6	.6		12.2	.6		2.4
5		3.0	82.0	1.8			12.0			1.2
6		3.2	77.0	.6	1.2	.6	13.5		1.2	1.9
7	.6	2.5	78.0	1.9		.6	12.0	.6	1.2	1.9
8	2.4	8.5	82.0	1.8		.6	1.2		1.8	1.8
9	3.8	20.5	67.0				5.1	.6	1.2	1.2
10	59.0	26.2	3.6	1.7			4.8		1.7	2.4

FIG. 11A

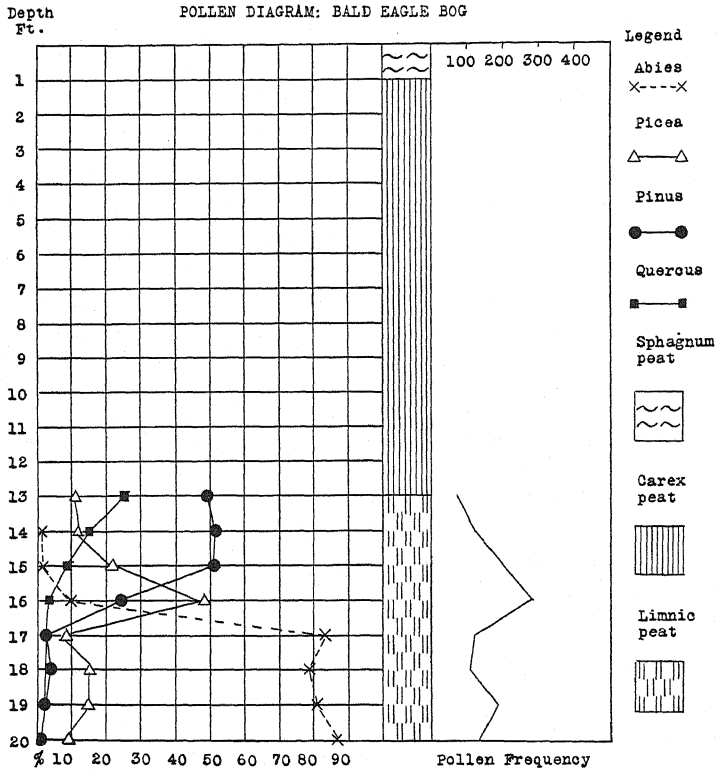


FIG. 10 B

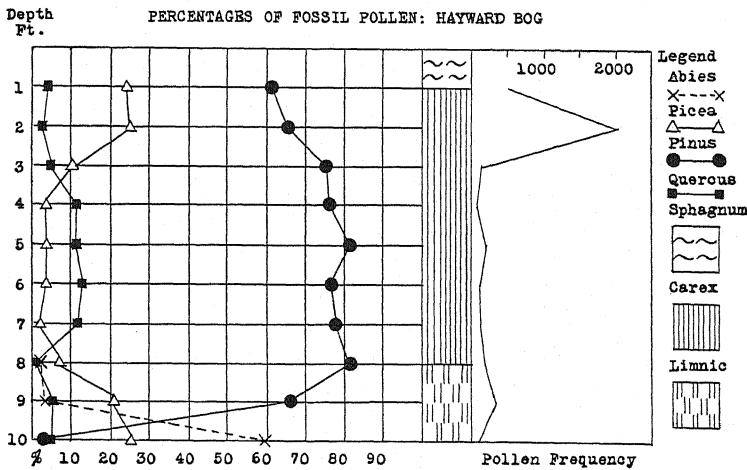


FIG. 11 B

PERCENTAGES OF FOSSIL POLLEN  
MISSION CREEK BOG

Depth Ft.	Abies	Picea	Pinus	Alnus	Betula	Fraxinus	Populus	Quercus	Salix	Ulmus
1		19.9	71.2		1.9			6.4	.6	
2		22.7	72.0		3.2			1.3		.6
3		28.3	64.5	.6	3.8			2.5		
4		33.0	62.0	.6	1.9			1.9		.6
5	1.2	36.2	54.0	.6	1.9			5.1	.6	
15	3.2	12.4	55.5	3.2	13.1	.6		1.3	.6	3.9
16	13.6	23.0	47.2	.6	6.8		.6	4.9		3.1
17	81.5	10.4	4.5		1.3		1.3	1.3		
18	94.0	4.4	.6					.6		

FIG. 12A

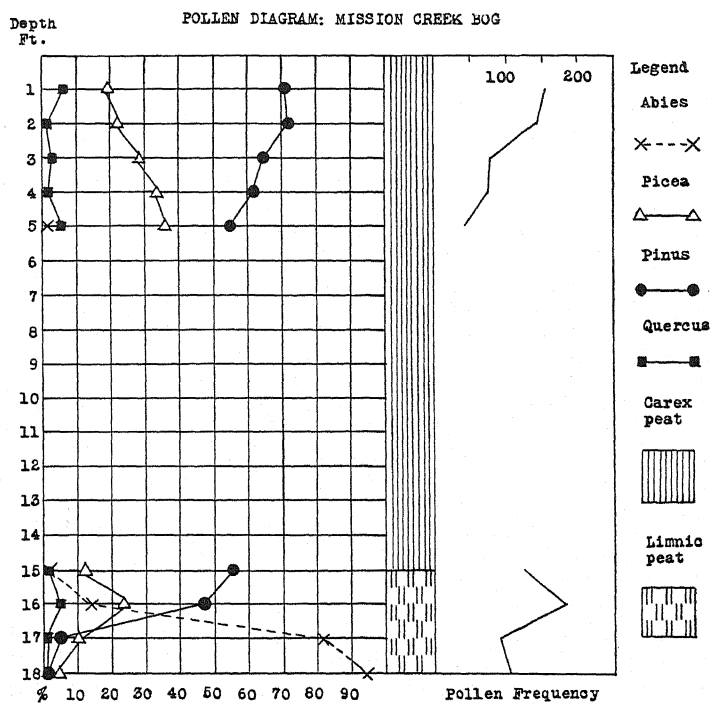


FIG. 12B

PERCENTAGES OF FOSSIL POLLEN: BAY LAKE BOG

Depth Ft.	Abies	Picea	Pinus	Acacia	Alnus	Betula	Coryla	Corylus	Ostrya	Populus	Quercus	Salix	Tilia	Ulmus
1		53.2	38.2		.6	5.5					1.8	.6		
2	.6	24.8	58.3		.6	11.4					2.5		1.2	
3	1.2	23.4	62.5		1.2	6.9					3.8		.6	
4	1.2	17.7	63.0		1.2	9.7					5.4		1.8	
5		15.5	38.6		3.6	38.6					1.8	1.8		
6	3.2	31.7	46.6		.6	11.1					3.8	1.9	.6	
7	1.9	28.8	43.0		1.2	19.8					4.4	.6		
8	.6	13.5	36.5		7.1	30.3					12.5		.6	
9	.6	11.5	43.0		2.4	14.6					20.5	2.4	3.0	1.8
10	.6	4.6	36.3		1.9	20.4					21.7	6.6	3.9	3.9
11		3.1	36.9		7.0	13.4					29.3	9.5		.6
12	.6	.6	25.8		9.4	18.8	.6		1.2		31.4	10.0	.6	.6
13		1.2	33.3		7.6	14.7					29.5	12.8		.6
14		1.9	28.4		5.2	18.5					36.4	7.9		1.3
15		3.1	44.5		3.1	6.3					29.0	9.4	.6	3.7
16	.8	8.6	34.5		4.3	9.5					30.0	8.6	.8	2.5
17		12.5	44.2		1.2	13.1	.6			.6	21.2	6.2		
18		7.9	57.8		1.9	11.8				.6	14.8	3.2		2.6
19	.6	4.3	73.5		2.5	5.6			1.8		3.7	4.3		3.1
20	.6	12.8	77.3		1.8	2.4			1.2	1.2	1.2	.6		.6
21	.6	10.2	66.0		3.2	10.2	3.2			1.2	1.2			3.8
22	2.4	21.5	48.5		2.9	16.1	1.2			3.6	1.8			1.8
23	19.9	28.4	17.0	1.7	3.9	20.4	2.2			2.2	2.8	.5		.5

FIG. 134

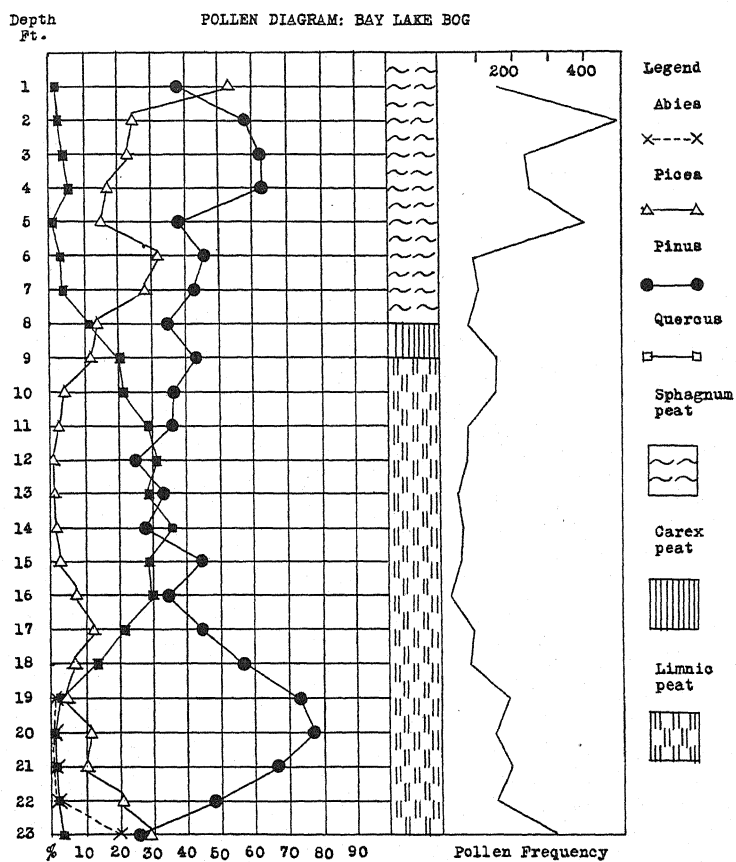


FIG. 13B



## PERCENTAGES OF FOSSIL POLLEN

## HIGHLAND BOG

Depth Ft.	Abies	Picea	Pinus	Alnus	Betula	Quercus	Salix	Ulmus
1	14.8	57.0	12.2	2.3	12.2	.6		
2	8.9	54.2	23.0	2.9	10.0	.5		
3	10.3	55.3	24.5	2.5	6.4	.6		
4	12.6	51.0	22.7		13.1	.6		
5	6.5	56.7	30.0	2.6	3.9			
6	5.0	54.0	37.0		2.5	.6	.6	
7	5.6	45.7	37.6	1.8	5.0	2.5	1.2	
8	1.2	42.5	45.0	1.9	7.6	1.9		
9	1.9	47.7	44.5	1.2	3.1		.6	.6
10	1.3	41.0	43.5	2.6	11.0	.6		
11	1.2	50.0	38.5	2.5	7.6			
12	.6	26.0	71.5		1.3	.6		
13		33.5	60.5	1.2	3.8	1.9		
14		28.2	59.0	3.1	8.1	.6		.6
15	1.2	27.0	62.0	3.0	4.3	1.2	.6	.6
16	1.1	30.3	53.7	2.2	8.5	.5	.5	2.8
17	.6	24.0	59.2	3.6	10.8	1.2	.6	
18	5.6	33.5	53.0	.6	6.8		.6	
19	5.1	47.0	38.0		6.3	3.2		
20	14.2	38.7	38.7		8.4			
21	5.5	35.0	48.0		9.8	1.2		.6
22	33.5	37.0	17.0		7.0	1.7	2.9	.6
23	30.2	32.5	26.5		4.8	1.2	2.4	2.4
24	35.0	27.0	20.4		4.7	2.7		
25	55.0	25.7	17.7		.6	.6		
26	58.5	19.4	21.3			.6		

FIG. 14A

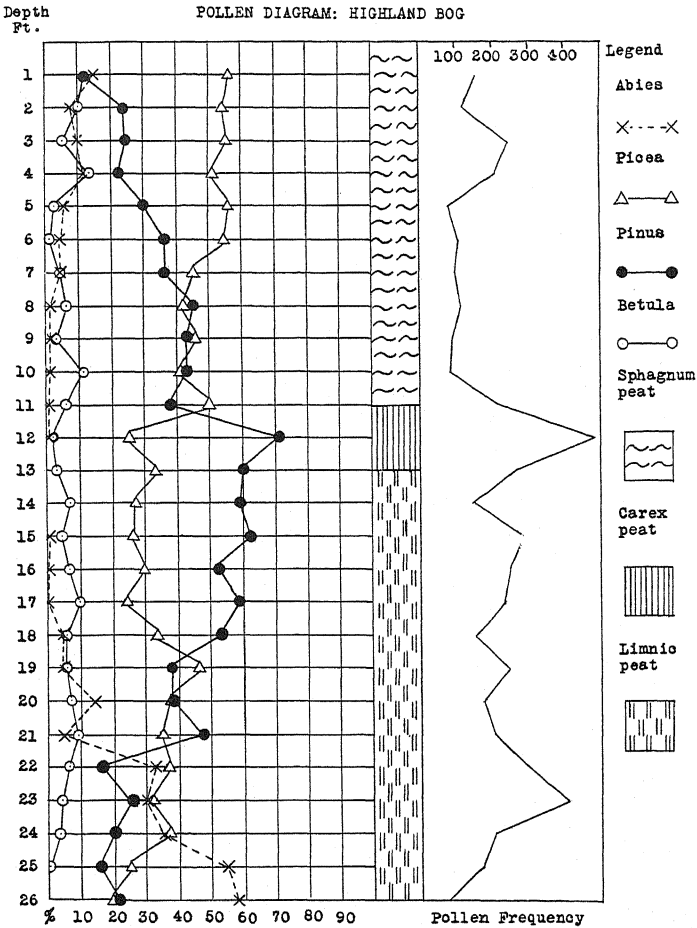


FIG. 14B

PERCENTAGES OF FOSSIL POLLEN: COLERAINE BOG													
Depth Ft	Abies	Picea	Pinus	Alnus	Betula	Corya	Carpinus	Ostrya	Juglans	Populus	Quercus	Salix	Ulmus
1	1.2	38.5	43.5	3.7	7.5					1.8	.6		.6
2	4.4	51.8	32.5	2.5	3.8		2.5			1.2			.6
3	1.3	54.5	37.5	3.4	2.0					.6			
4	1.8	53.0	25.8	6.6	6.6		3.1			3.6			
5	3.4	56.0	23.1	3.4	11.0		1.1			1.1		.5	.5
6	3.1	58.8	34.0		1.8					1.8			
7	8.2	59.9	25.5	1.2	3.8		.6			.6			
8	1.9	42.5	39.9	3.9	7.3					3.9			
9	.6	42.2	51.0	1.8	2.4					1.8			
10	1.3	32.6	57.1		2.6					4.0	2.0		
11	1.9	23.0	61.0	1.9	2.5		1.2			8.3			
12	.6	20.4	70.0	1.9	4.4					2.5			
13	.6	37.5	58.0		2.0					1.3			
14	.6	22.4	64.0	.6	2.5		.6			3.8	3.8		
15	.7	15.6	69.0		1.4		.7	1.4	.7	5.9	3.7		.7
16	.6	15.2	72.0		.6	.6	1.2		.6	9.1			
17	.6	16.6	73.0		2.5					2.5	3.8		.6
18	.6	18.0	71.5		3.2					2.5	3.2		.6
19	3.7	26.0	60.0		4.9		1.2	.6		1.8			1.2
20	8.5	26.0	61.0		.6		1.3			1.3			1.3
21	25.6	33.5	20.7		12.8					.6	2.4	1.8	2.4
22	32.7	21.0	23.6	1.3	9.8					1.9	7.2		1.9

FIG. 15A

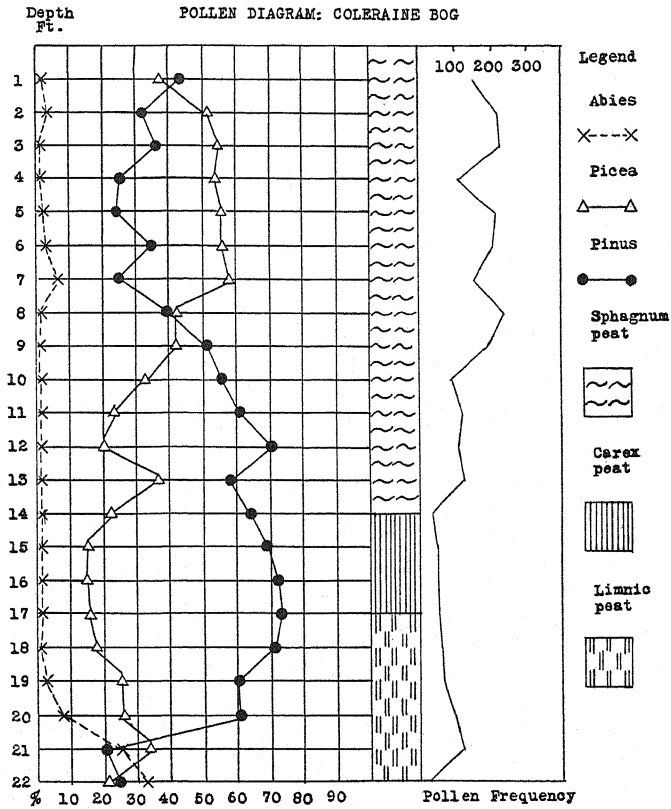


FIG. 15B

The pollen diagrams of the two northernmost bogs, Coleraine (fig. 15) and Highland (fig. 14), reveal the predominance of *Abies* while the lowest levels were being deposited, and its pollen is found in lower percentages at practically all levels upward. The *Pinus* curve culminates near the center and decreases toward the surface, while the *Picea* curve remains rather constant from the bottom to the center of the bogs and then gradually increases. *Quercus*, in contrast to the older and more southern bogs, was always less than 10 per cent at all levels in both bogs.

### Conclusions

When the Late Wisconsin ice sheet reached its maximum extension in Illinois, the conditions controlling the flora of the region south of the ice edge were probably as pictured by TRANSEAU (35), WOODARD (43), and others. South of the Valparaiso moraine the country was evidently comparatively flat and dissected by several pre-Late Wisconsin streams. While the ice sheet remained stationary and formed the Valparaiso moraine, most of Illinois, because of climatic conditions, was occupied by tundra as far south as the southern part of Williamson County. East of Illinois the tundra, owing to differences in topography, gradually became narrower whereas west of Illinois it was probably much wider.

Immediately below the tundra, between the Appalachian and Ozark mountains, where temperate conditions and abundant rainfall prevailed, there was probably a mixture of conifer and deciduous forests. These forests, like all other biota, had previously been forced to migrate southward by the advancing glacier. Probably a definite conifer belt existed next to the tundra, which, according to ADAMS (5), may have reached the latitude of the Ohio valley, with extensions farther south at higher elevations.

In the southeast the edaphic and climatic conditions for the support of a deciduous forest were ideal (4), and as ADAMS (5) states: "This area has been important not only as a region of preservation, but also as a center of origin. Here is found the best development of the deciduous forest . . . of North America."

With the decline of the Late Wisconsin ice sheet, many modifications in topography were brought about by the waters from the melt-

ing ice. New drainage channels were formed and others filled with drift. Many depressions were also formed which later aided in the dissemination of plant life. As the Late Wisconsin ice receded, it deposited drift which was far more hilly than that of the Early Wisconsin, and this later served as an excellent environment for the invading forests.

With an amelioration of climate, there naturally followed a slow migration of plant and animal life, the rate and type of succession depending upon climatic, edaphic, and topographic conditions. The edaphic effect has been emphasized by FULLER (17), WOODARD (43), DE FOREST (11), WILDE (41), and the topographic by FULLER (18). The tundra plants closely followed the edge of the retreating ice, and as their remains accumulated and became part of the soil, they paved the way for the succeeding biota.

Several drainage channels, the most important being the Mississippi, Rock, Illinois, and Wabash rivers, served as pathways for the invading forests from the south. All pollen diagrams indicate that the conifers *Abies* and *Picea* were the first dominant trees to appear on the newly uncovered land. As the climate became warmer and edaphic conditions changed, the oaks, maples, etc., gradually invaded and superseded the conifers on the uplands (43, 8). Along the streams the pioneers were probably similar to those under like conditions today (9).

The results of the pollen analyses of the eleven bogs found within the limits of substage III of the Wisconsin epoch agree in many respects with those of SEARS (31, 32) and AUER (2). Reporting on his study of 28 bogs, AUER states: "In the bottom layer of peat bogs in southeastern Canada, *Picea* and *Abies* pollen appear in abundance, but their curve, a little higher up, rapidly drops to a minimum, when the pollen amounts of the hardwood reach their highest value. In the surface parts the pollen amount of spruce trees again increases, whereas that of hardwood is decreasing."

The northern bogs, Coleraine and Highland, which are younger than those of substage III of the Wisconsin, show that the type of vegetation has remained practically the same throughout their development. This may be attributed chiefly to climatic conditions.

The pollen diagrams of the bogs found upon the drift of substage

III indicate that climatic conditions remained very uniform during the period represented by the upper two-thirds of the diagrams. Likewise no indications of climatic fluctuations are seen in the diagrams of the substage IV bogs. These studies do not show evidence of such climatic fluctuations as those found in Europe and designated in the Blytt-Sernander hypothesis (44). They also do not seem to afford any data that would support the hypothesis of SEARS (33) that five or six variations of climate have occurred in postglacial times.

### Summary

1. Taking into consideration all the limitations of pollen analysis, the fossil pollen counts of fourteen bogs in Illinois, Wisconsin, and Minnesota show the succession of the forests during their entire development.

2. Eleven bogs are found within the limits of substage III of the Wisconsin epoch, and three within the limits of substage IV.

3. *Abies* and *Picea* pollen were the predominant ones at the lowest levels in all bogs.

4. Dryness and decay of peat often renders it unfit for pollen analysis.

5. The bogs of substage III reveal a succession from *Abies-Picea* forests to deciduous ones, the oaks being the predominant trees of the latter. The change from *Abies-Picea* to deciduous forests is generally abrupt, and if the thickness of peat is considered an indicator of age, the *Abies-Picea* period, especially in the southern bogs, was shorter than the deciduous period. *Quercus* pollen was more abundant in the southern bogs.

6. The younger northern bogs belonging to substage IV show that the conifers have been the significant trees throughout their history.

7. Climatic conditions remained very uniform throughout the period represented by the upper two-thirds of the pollen diagrams.

8. The results do not correlate with the Blytt-Sernander hypothesis.

Grateful acknowledgments are due to Dr. GEORGE D. FULLER of the University of Chicago for his careful supervision and thoughtful criticism during the course of this study; to Dr. GUNNAR ERDTMAN

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# ANATOMY OF THE VEGETATIVE ORGANS OF THE PARSNIP

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 455

WINIFRED CAROLINE WARNING

(WITH FORTY-FOUR FIGURES)

## Introduction

Apparently no study of the anatomy of the parsnip has been reported, although descriptions of various tissues and organs of other Umbelliferae appear in the literature. HOFFMAN (7) and COURCHET (3) have studied the gross morphology; TRECUL (8), vessels; TRECUL and VAN TIEGHEM (9), secretory canals; VAN TIEGHEM and DULIOT (10), origin of the first group of secondary roots; and HOAR (6), stem anatomy. In some of these contributions references to the structure of the parsnip are found, but a connected study of its anatomy does not appear.

MATERIAL AND METHODS.—The material was obtained largely from seedlings and plants grown in the University of Chicago garden and partly from seedlings grown in the greenhouse. The variety used is known commercially as Hollow Crown.

Three methods of fixing were employed, Flemming's medium solution, chromo-acetic solution, and formalin-acetic-alcohol mixture. The safranin, gentian violet, orange G combination, and Haidenhain's iron-haematoxylin were used in staining.

## Gross morphology

*Pastinaca sativa* L. is normally a biennial producing a large taproot in which much food is stored, a stem, and leaves during the first year; and leaves, a floral axis, flowers, and fruit during the second year.

The mature root is conical in shape and represents the primary root together with the hypocotyl. Four rows of shallow depressions occur on it, running almost directly downward, and secondary roots are diverged in these depressions. The stem is short and from it

diverge numerous leaf bases which are placed in a close spiral. The cotyledons are opposite but all other leaves are alternate. Juvenile leaves are simple while those produced later are compound or decomposed. The blades of simple leaves vary from obovate with entire margins in the cotyledons to reniform with crenate margins and deeply three-lobed with toothed margins, while in the leaflets they range from obovate with crenate margins to deeply incised with toothed margins. All are net-veined with branches diverging from the prominent midrib, separating each leaf or leaflet into definite, rather small vein islets. The leaves are large and there is a lack of uniformity in both color and texture. They may vary from deep olive to light green in the blades, while the petioles range from dark to light or reddish green and may be coarse or fine and rather smooth or rough. The floral axis is coarse, columnar, deeply five grooved and branched, and bears numerous flowers as well as leaves. These present an increasing and decreasing series in the number and arrangement of leaflets involved. On the lower portion of the axis they are odd pinnately compound. Proceeding in the acropetal direction, they are decomposed, then compound, and in the upper level vestigial.

### Anatomy

A longitudinal section through a mature root shows an outer, rather thin layer of periderm and beneath it a heavy layer of phloem, consisting of phloem, phloem parenchyma, and fibers. Centripetal to this is a narrow layer of cambial cells, and at the center the xylem composed of vessels, fibers, and xylem parenchyma. In the upper portion or region of the hypocotyl a core of pith is differentiated and lies within the xylem. If a section is cut where secondary roots have been diverged, radiating strands of vascular tissue may be traced running outward and defining the position of these roots. The stem is greatly constricted in diameter and shows the same type and arrangement of tissues as are developed in the hypocotyl, but the pith has become much broader and consequently the other tissues form a very narrow band composed largely of leaf traces. A longitudinal section of the floral axis shows an epidermis, collenchyma, cortical parenchyma, a band of bundles, and a pith hollowed at the internodes. Since many of the tissues in the various organs

are similar in structure, their anatomy may be discussed under one caption.

Pith is differentiated in the hypocotyl, stem, and floral axis, and consists of large thin-walled cells with small intercellular spaces. In transverse section the cells are roughly hexagonal in outline and the intercellular spaces usually triangular, rarely rectangular. In longitudinal section they are rounded rectangular, one to two times as long as wide, with transverse walls, so that the pith is composed of cells which are somewhat barrel-shaped with hexagonal bases (figs. 19, 26).

Xylem is developed in all the vegetative organs and consists of fibers, vessels, and xylem parenchyma. The vessels are annular or spiral in the primary tissue and scalariform or reticulate in the secondary. Large vessels are porous with transverse cross walls which are sometimes somewhat oblique, while the smaller ones are pointed but porous. The xylem parenchyma cells are thin-walled and, as seen in transverse section, are hexagonal in outline when young but become much distorted as they enlarge, presenting various forms (figs. 33-41). In longitudinal section they are elongated and pointed at the ends. The walls are pitted, both simple and bordered pits occurring, depending upon the age of the cells.

Phloem develops in all vegetative organs and is composed of sieve tubes, companion cells, phloem parenchyma, and fibers (figs. 2, 42). The sieve tubes as seen in transverse section are small, thin-walled, and hexagonal in outline; in longitudinal section they are greatly elongated with sieve plates on the sides and ends, and are essentially rectangular with transverse walls. The companion cells are as long as the sieve tubes and in transverse section are pentagonal, quadrangular, or triangular. The phloem parenchyma is thin-walled, roughly hexagonal in cross-section, and a polygon of from four to nine sides in longitudinal. The cells are therefore rather regular polyhedrons with faces varying in number within a narrow limit, and are practically isodiametric. Those near the periphery of the root and hypocotyl are much smaller and heavier-walled than are the cells farther removed.

Collenchyma occurs in the stem, floral axis, and leaf, and the groups are usually reniform in outline and consist of cells with heavy

walls and much heavier thickenings at the corners. In transverse section the cells are hexagonal when young but become rounded as the walls thicken, always showing prominent round nuclei; in longitudinal section they are several times longer than wide, with cross walls transverse or oblique and the nuclei broadly lenticular (figs. 22, 26, 31).

Cortical parenchyma is differentiated in the petiole and floral axis and is of two types, an outer zone of areas beneath the epidermis and an inner zone. The subepidermal groups of cells are photosynthetic, all of them containing numerous chloroplasts. The walls are thin and in transverse section the cells are small and roughly hexagonal in outline; in longitudinal section they are slightly rounded and somewhat longer than wide, with essentially transverse walls at intervals bounding intercellular spaces which are connected with stomatal cavities. The inner, non-photosynthetic zone consists of large, thin-walled cells which in cross-section are hexagonal with small intercellular spaces. In longitudinal section they are rectangular, much longer than wide, with transverse walls (figs. 22, 26).

The cortex of the root, hypocotyl, and stem resembles the cortical parenchyma and in cross-section consists of thin-walled cells, hexagonal in outline, with small triangular, rarely quadrangular intercellular spaces which are not distinguishable when the tissue is young, but are apparent when it becomes older (fig. 1). In longitudinal section the cells are rectangular and almost isodiametric, with transverse walls.

Sclerenchyma in the form of fibers occurs in all the vegetative organs. In transverse section the cells are small, heavy-walled, and hexagonal in outline; in longitudinal section they are narrow and much elongated, with pointed ends. Normally in the root and hypocotyl they are not lignified during the first year but become very woody during the second; in the other organs they lignify early, this being particularly true of the floral axis (figs. 22, 26, 42).

The epidermis of the different organs varies somewhat according to its several functions. That developed on the primary root, stem, and hypocotyl consists of a single layer of practically isodiametric cells with a somewhat rounded outer wall, the cells being broadly rectangular in longitudinal section. The epidermis of the petiole,

veins, and floral axis is similar in transverse section and consists of a single layer of heavy-walled, essentially isodiametric cells with collenchymatous thickenings in the corners; in longitudinal section it consists of slightly rounded cells three to four times as long as wide, with transverse walls; in face view the cells are tabular (figs. 22, 24, 26, 27). The epidermis, which is differentiated on the portions of the leaf blades between the veins, differs in some respects from that covering the veins. In transverse and longitudinal sections its cells appear similar in outline to the tabular, but in face view the walls are tortuous (figs. 24, 25).

Periderm is differentiated on the root, hypocotyl, stem, and regions which have been injured, and is initiated after the sloughing of the outer tissues in the root and hypocotyl, while in the stem it follows the desiccation of the leaf bases. The cork which develops on the root and hypocotyl is very regular. In transverse section it consists of closely packed, thin-walled, rather small rectangular cells from two to three times as long as broad, and in longitudinal section it is rather similar (figs. 5, 6, 32). The periderm which develops on the stem and places of injury consists in cross-section of heavy-walled, rounded, almost isodiametric cells.

Cambium is differentiated in all the vegetative organs and in transverse section is composed of small, thin-walled, closely packed, fairly rectangular cells with round nuclei. In longitudinal section the cells are much longer than wide, with transverse or sometimes somewhat oblique walls and having elongated lenticular nuclei containing one or two prominent nucleoli (figs. 43, 44).

The oil ducts which are cut off from the pericycle and primary phloem are described under the discussion of ontogeny. Other canals are differentiated in the phloem parenchyma, phloem of bundles, pith, and cortical parenchyma. In transverse section the duct is a polygon of varying number of sides, while the epithelial cells are usually pentagonal and smaller than the adjacent cells. In longitudinal section the canal is much elongated and at times interrupted. Its roughly rectangular epithelial cells vary from isodiametric ones to those several times longer than wide and are characterized by large, rounded nuclei and dense protoplasm (figs. 7, 9, 10). Oil ducts rarely anastomose, but this may occur when tissues are reoriented or when cells are rapidly increasing in size, as in the petiole and root.

Two canals may be oriented so that their epithelial cells touch, and in case the intervening walls break down, a canal is formed which is elliptical in cross-section and much broader than usual in longitudinal section.

Striations are developed on all tabular cells and in transverse section are short triangular projections consisting of minute conical protuberances, transparent and somewhat iridescent, lying in longitudinal rows which are more or less parallel and occur at intervals of from seven to twelve striations per cell (figs. 21, 22, 24, 26).

Hairs occur on tabular cells on the ridges of the petiole and floral axis, and on the veins and margins of leaves. They are of two types, a straight, slender, conical form and a shorter, curved, heavier-walled type (figs. 29, 30). Both forms are transparent and iridescent with a large nucleus near the center. Their surfaces are closely beset with the same type of conical protuberances which form the striations, and it seems probable that these protuberances as well as the hairs may be responsible for the skin irritations which result from handling parsnip leaves, as reported by BLANCHARD (2) and others. The number of hairs per unit area varies with the age and size of the organ on which they are developed.

Stomata are differentiated on the stem, leaf, and floral axis. The stomata of the petiole and floral axis are confined to the grooves and are very numerous (figs. 27, 28). Naturally few are developed on the sheathing portion of the leaf bases, and on the blade they are limited to the cells with tortuous walls where they occur on both sides of the leaf, being somewhat more numerous on the lower than on the upper surface, and less widely separated in young than in older tissue. The stomata are of the common mesophytic type with guard cells which contain many plastids. The stomatal cavities vary in size, depending upon the organ in which they are differentiated, being small in the stem, floral axis, petiole, and cotyledons and much larger in the blade (figs. 21, 22, 24, 25, 26). Relatively few appear on the stem, and those which persist over the growing season develop into lenticels which are of the ordinary type, with a heavy periderm, ramifying intercellular spaces, and a relatively small number of complementary cells. The closing cells break down early in the spring of the second year and the lenticels gradually disintegrate.



### Ontogeny

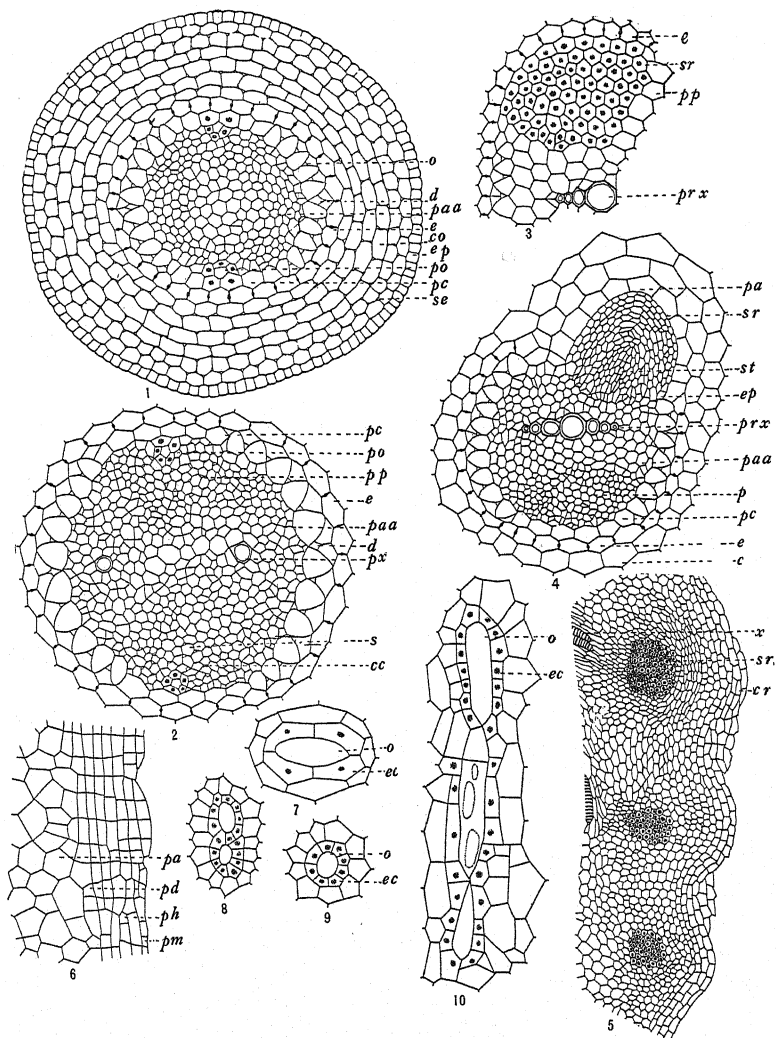
Three regions appear in the young seedling: root, hypocotyl, and cotyledons. The junction of the root and hypocotyl is readily distinguished, being marked by an enlargement of the hypocotyl and an abrupt constriction of the root. Small ephemeral root hairs develop and secondary roots are diverged. The stem is not distinguishable in the earliest stages, and the cotyledons are small.

#### PRIMARY ROOT AND HYPOCOTYL

The primary root falls under JANCZEWSKI's third type, *plerome* and *periblem* defined with *periblem* overlying the *plerome*, while *calyptragen* and *dermatogen* are differentiated together.

Since the seed is slow in germination, many of the earlier stages must be studied while the plant is still within the fruit coat. A transverse section through the proximal part of a root which has been in process of germination for three days shows a mass of undifferentiated cells, and at four days the epidermis consisting of a single layer of cells is differentiated (fig. 1). At five days the pericycle is readily distinguished. In transverse section it is composed in its earliest stages of a single row of isodiametric, hexagonal cells much larger than any of the others, while longitudinally the cells are broadly rectangular. The cortex, consisting of from seven to ten rows of relatively large cells, is thus set off from the central meristem, and the endodermis, composed of a single row of cells, isodiametric and hexagonal and smaller than those of the pericycle, is distinguishable. In longitudinal section the latter are rectangular and little longer than wide, and because of the dense protoplasmic content the Casparian strips, which are never very heavy, are not easily distinguished. When the cells lose their protoplasm, however, they are seen to consist of radial thickenings with a small lenticular expansion at the center of the wall (figs. 1, 2). During the fifth and sixth days the cells of the pericycle divide rapidly, and the resulting walls may be oblique, tangential, or vertical. The vertical and obliquely longitudinal divisions serve to increase the number of cells in the pericycle, the tangential divisions producing endodermal cells and the oblique ones usually forming oil ducts.

The first secretory canals which are differentiated and in which



FIGS. 1-10.—Fig. 1, transverse section of young seedling root showing early stages of primary tissues with pericyclic oil ducts differentiated and phloem oil canals abutting on pericycle,  $\times 220$ ; fig. 2, cross-section through young hypocotyl showing protoxylem points, sieve tubes, companion cells, and phloem oil ducts one cell removed from pericycle,  $\times 220$ ; fig. 3, transverse section of primary root with meristem of secondary showing position of root, primary xylem, and unruptured endodermis,  $\times 440$ ; fig. 4, cross-section showing older secondary root with endodermis partially disintegrated,  $\times 220$ ; fig. 5, transverse section of older root showing origin of second and later groups of secondary roots in pericyclic periderm,  $\times 220$ ; fig. 6, longitudinal section of periderm,  $\times 220$ ; fig. 7, transverse section of oil ducts, primary type,  $\times 440$ ; figs. 8, 9, some types found in secondary tissues,  $\times 110$ ; fig. 10, longitudinal section of oil duct,  $\times 110$  (cc, companion cell; co, cortex; cr, cork; d, central oil duct; e, endodermis; ec, epithelial cell; ep, epidermis; o, oil duct; p, phloem; paa, primary parenchyma; pc, pericycle; pd, periderm; ph, phellogen; pm, phellem; po, phloem oil duct; pp, primary phloem; prx, primary xylem; px, protoxylem point; s, sieve tube; se, intercellular space; sr, secondary root; st, stele; x, xylem).

pericyclic cells alone are involved are formed in the following manner: Beginning with the central pericyclic cells, which are located at points  $180^\circ$  apart, from two adjoining sides of two adjacent cells quadrilateral sections are cut off. Divisions follow on both sides of the central cell, quadrilateral sections are cut off (to the right on one side and to the left of the central duct on the other) until two arcs of from 11 to 15 cells are formed on opposite sides of the root. Schizogenous splits develop as mitosis proceeds, and the central canals develop. In transverse section these are quadrangular, being bounded by two of the small quadrilateral and two of the larger hexagonal cells which function as epithelial cells. All other pericyclic ducts are triangular, being limited by one of the small quadrilateral and two of the larger hexagonal cells, and a gradual diminution in size from the center to the end of the arcs is apparent (figs. 1, 2). The number of epithelial cells bounding the pericyclic oil ducts seems never to be increased by mitosis, and consequently these canals remain distinct from those which are differentiated later. The protoplasm of the epithelial cells is dense at first and the nuclei are prominent, but as the cells grow older they gradually grow faint and the ducts probably become functionless. In longitudinal section the epithelial cells are rectangular and isodiametric, and no anastomosis of pericyclic oil ducts was observed. There are two groups of from four to five pericyclic cells lying centrifugal to the primary phloem which do not divide to form oil canals, retaining their hexagonal outline (figs. 1, 2). The order of pericyclic cell division just described has been adopted for the sake of convenience. While the central cells are usually the first to divide, exceptions occur, and individual cells or one of the arcs may divide irregularly or be retarded in division. Development of the pericyclic oil duct was first discussed by TRECU (8), and the results of this study differ from his conclusions in no essential respect.

Concomitant with the development of the pericyclic oil canals is the differentiation of the primary phloem. Two protophloem points,  $180^\circ$  apart and located opposite the middle of the non-secretory pericyclic cells (and removed from them by two or three cells in the centripetal direction), enlarge and mitosis follows, in which small cells are cut off, thus differentiating the first sieve tubes and com-

panion cells. Divisions follow rapidly; protophloem is followed by metaphloem; and two groups of primary phloem are developed which are elliptical in outline and limited by the pericycle and parenchyma.

While the metaphloem elements are still in process of differentiation, two cells  $180^{\circ}$  apart, abutting on the pericycle and near the center of the protophloem, gradually lose their protoplasm through the disappearance of the comparatively small nucleus, followed by the cytoplasm, leaving a clear lumen. The cells which are adjacent to them, two pericyclic and three phloem, now become densely protoplasmic and the central cells function as oil canals while the adjacent ones become epithelial (fig. 1). Infrequently two adjoining cells lose their protoplasm but the wall separating them fails to disintegrate and two adjacent oil ducts are thus formed. These phloem ducts function for a short time only, and usually cannot be distinguished after the cortex has disintegrated.

Before the metaphloem has fully matured, two procambial cells, lying directly opposite the central oil ducts and separated from them by two or three parenchymatous cells, are differentiated from the central meristem and mature into the first elements of the protoxylem. Development of the primary xylem proceeds centripetally, until the metaxylem cells meet at the center to form the diarch xylem plate which consists of from seven to nine cells and contains fewer cells than the primary phloem. The primary xylem is surrounded by parenchyma which separates it from the other tissues.

There are no marked differences between the epidermis, cortex, endodermis, and pericycle of the primary root and hypocotyl, except that the number of cells in each tissue in the latter is greater, resulting in all probability from the increase in diameter. The phloem of the lower portion of the hypocotyl is similar to that of the root, but in the upper levels the phloem oil canals are differentiated one cell removed from the pericycle, while in the root they abut directly upon it and the intervening cells are of pericyclic origin (fig. 2). Two protoxylem points appear as in the root, and these are rapidly followed by others until two groups of from four to five cells are developed. In the root centripetal growth completes the xylem plate, but in the hypocotyl this does not take place because the

procambial cells fail to mature into xylem but remain parenchymatous, and this central parenchyma by cell division and growth develops into the pith (figs. 12-14, 19). The change in orientation of the xylem and phloem of the hypocotyl is discussed under the section on transition.

#### TRIARCH CONDITION

The primary xylem of the root and hypocotyl is normally diarch, although a few exceptions appeared, for in the several thousands of plants which were studied seven were found with a portion of the primary xylem triarch and in every case triple cotyledons developed. Five of these plants were available for close study, one in the initial and four in more advanced stages of growth. Since no discussion of such structures was discovered in the literature, and since triarchs have been tested for heritability of the arch condition, a brief statement concerning the anatomy seems desirable. It is freely admitted that absolute conclusions cannot be deduced from so small an amount of material, but in any species the condition seems to be of rare occurrence and in all probability a number of species must be studied to arrive at a satisfactory explanation.

In the initial stage, or when the plant is still within the fruit coat and there exists no definite demarcation between root and hypocotyl, the triarch structure appears at a considerable distance above the root tip and in all probability is restricted entirely to the hypocotyl. The pericyclic and phloem oil ducts as well as the protoxylem points are differentiated in their normal order, number, and position in the root, but one protoxylem point appears in advance of the other. As the level of the hypocotyl is reached and soon after the second protoxylem point has developed, a third phloem oil canal is differentiated. This appears a short distance above the level of the xylem point and is separated from one of the first two phloem oil ducts by four or five cells.

These four or five intervening cells and those adjacent to them centripetally, as well as the non-secretory pericyclic cells located centrifugally, soon become densely protoplasmic and rapid cell division follows, causing the portion of the stele involved to bulge slightly into the cortex. Concurrently with these changes there occurs a wider separation of the phloem oil ducts and a crowding of the peri-

cyclic oil canals and the protoxylem points, so that eventually they appear on an arc approximately  $120^{\circ}$  apart. A third central pericyclic oil canal and about one half of the normal number of pericyclic oil ducts are now differentiated on the longer arc midway between the other two. A third protoxylem point is developed at a level considerably above the third central oil duct, and three cotyledons appear which are normal in all respects with the exception that they lack one oil duct.

Four plants in a later stage of development were studied, and in all of them the primary xylem remained diarch throughout the extent of the root and the triarch structure became apparent in the lower portion of the hypocotyl, at the level where the bundles were being differentiated before transition. One bundle was perfectly normal, but as the bifurcation was about to be formed in the other, the two xylem arms swung apart and were oriented at right angles to each other with one arm following the bifurcation curve and the other extending in a radial direction, thus forming the triarch.

Development of the oil canal did not coincide in the four hypocotyls, for in three of them there were three groups of pericyclic oil ducts, two containing the normal number of cells and one with fewer than usual, while in the fourth there were two groups of perfectly normal oil ducts. In this particular case a group of phloem cells was differentiated centripetal to the pericyclic oil canals, a most unnatural position. A third phloem oil canal could not be distinguished in any of these roots, but these ducts are ephemeral and soon become unrecognizable. The transition followed the usual lines, except that the halves of the split bundle did not form a normal bifurcation (fig. 19). The cotyledons were normal with two exceptions: in one no oil ducts accompanied the lateral bundles and in another one lateral bundle did not divide until the middle of the cotyledons was reached, thus causing two of the cotyledons to adhere up to this point. In two of the hypocotyls secondary roots were differentiated while transition was in progress, and three of the plants were belated in germination.

It is evident that the abnormal structure in the plants studied was largely if not entirely confined to the hypocotyl, for in every case the root was diarch throughout practically all of its extent, and this coincides with HILL'S (5) work on *Piper*. The third xylem arch in

each plant is always the result of splitting instead of bifurcation during transition, and various irregularities follow, differing in each plant. It seems apparent that the so-called triarchs are not triarch but diarch, in which one bundle is split at or near the time of transition; and this agrees with the results of plant breeders who have found that the triarch structure is not heritable. The cause of the abnormality is difficult to trace, but it is possible that differentiation of secondary roots in the hypocotyl and belated germination may be contributing factors. That true triarchs were not found does not in any way assume that they do not occur; but if they appear a cursory examination should reveal their structure, for six groups of secondary roots should be differentiated and a section near the root tip should show the triarch xylem plate. Moreover perfect triple cotyledons should be diverged and naturally no irregularities in structure would be expected. It is again emphasized that no definite conclusions can be drawn from the few cases studied; this report is merely a contribution to clear up a puzzling situation which seems to have been misinterpreted.

#### LATERAL ROOTS

The lateral roots are of two types, permanent and transient. The former are differentiated in the pericycle of the primary root before disintegration of the outer tissues. They persist throughout the life of the plant and are few in number. The transient laterals are also of two types, the first of which is initiated in the same manner as the permanent ones but persists only until the cortex sloughs; the second is differentiated in the pericyclic cork during any active period and is of short duration.

Since the primary root is diarch, the secondary roots are developed at points which are located near the limits of the non-secretory pericyclic cells; but at any level only one root is initiated. When a root of the permanent or of the first type of transients is about to be differentiated, a single cell of the pericycle becomes meristematic, enlarges, and mitosis follows. In cross-section the first cell divisions are tangential, followed by radial and tangential ones and then those in various directions, and the root is developed following the lines of the primary. As it enlarges it ruptures the endodermis, cor-

tex, and epidermis, and as it proceeds apparently absorbs two or three layers of cortical cells and a connection is made with the primary xylem (figs. 3, 4). The second type is initiated in groups after the endodermis, cortex, and epidermis have disintegrated and secondary tissue has developed, and is differentiated near points where transient roots sloughed, thus leaving the conductive tissue free. The phellogen in these regions becomes meristematic and there is an unusual proliferation of periderm, followed by development of initial cells in the phellogen in proximity to the conductive strands. The development of these roots does not differ from that of the secondaries, except that they rupture the periderm as they grow outward (fig. 5). The permanent and first type of transient laterals are endogenous in origin, but the second type of transients can be considered so only in the sense that the periderm in which they are initiated is derived from the pericycle, which was endogenous although the cork is peripheral and a part of the secondary body when these roots are differentiated.

The number of lateral roots which may be developed in association with the free conductive tissue of a secondary one depends upon the size of the conductive strand. Since this gradually increases, the number during the first year is much smaller than during the second year when fascicles of over one hundred are frequently developed. While some of these diverge from the lower part of the main root, by far the greater number appear on the upper portion. In general structure laterals are similar to those of the primary root, but the cells of the phloem and parenchyma are relatively fewer in number while those of the xylem are greater. The arch condition of the first group of laterals usually conforms with that of the primary, but because of the excessive xylem there is a crowded condition, since fourteen or more cells are involved and the xylem frequently abuts on the pericycle. Two or three more or less parallel rows of rather perfect triarch or tetrarch roots may appear.

#### STEM

The structure of the stem is similar to that of the hypocotyl until the lateral cotyledonary traces are differentiated and the cotyledonary plate defined. The growth of the traces, together with the re-



orientation of bundles, forces the pericyclic oil ducts outward, then separates them into groups and single canals, and finally they become indistinguishable. The endodermis is ruptured, and concomitantly the tissues lying centripetal to the bundles become meristematic and leaf traces are rapidly developed, thus setting off the pith. When the tissues are young the bundles are discrete but become crowded as leaf traces continue to be differentiated. The cortex is relatively narrow, consisting of from six to seven rows of cells when the plant is young, and gradually increasing as it grows older. The stem is shallowly five-grooved, and colenchyma is developed in the ridges and hairs on the surface when young, disappearing as the leaf bases are diverged. The upper concave portion remains meristematic during the winter and is protected by leaf bases and covered with small young leaves which, because of their close packing and dense covering of hairs, are frost resistant. In the spring these develop into basal leaves.

#### COTYLEDONS

The short petiole of the cotyledons is reniform in transverse section when young, but becomes slightly three-lobed on the abaxial side as it grows older. It is from seven to eleven cells in thickness, consisting of a single layer of epidermal cells and a cortical parenchyma which is traversed by three bundles whose origin is discussed under transition. One large oil duct separated from the phloem by a few parenchyma cells is associated with each of the bundles, and a smaller canal is developed near the xylem of the median bundle (fig. 16). A single row of epidermal cells is differentiated on the blade, and below it on the upper side a layer of rounded palisade cells appears. The spongy mesophyll lies directly beneath and all palisade and mesophyll cells are densely filled with chloroplasts. The three bundles which enter the blade branch at various intervals, and as they ramify one oil duct is developed with each branch. The cotyledons persist only until four or five leaves appear, when they are overshadowed and desiccation follows.

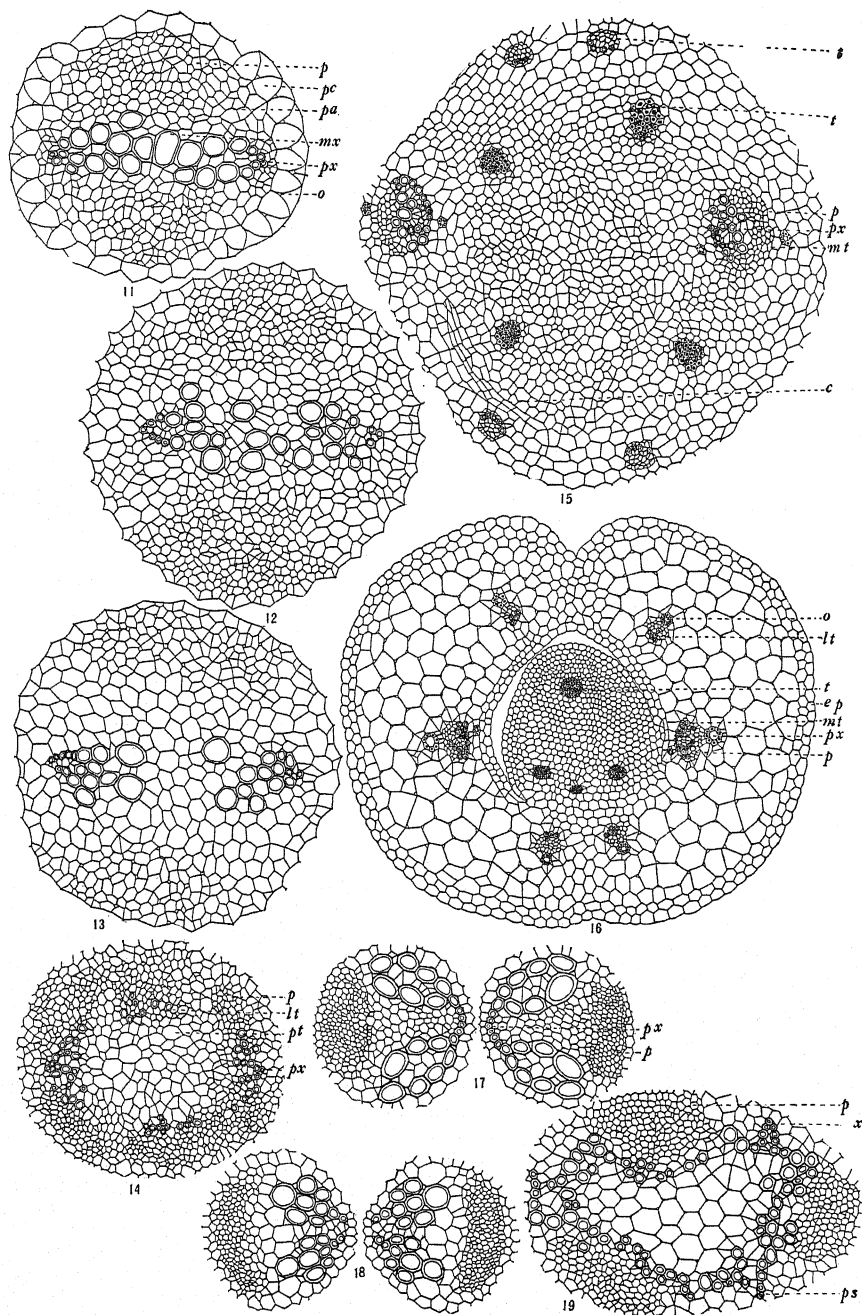
#### EXARCH-ENDARCH TRANSITION

The transition region is initiated in the lower portion of the hypocotyl and terminates in the petioles of the cotyledons. There is a

gradual and simultaneous reorientation of the elements involved, until they appear on a curve  $180^\circ$  from their original position. The first evidences are noticeable when a narrow bifurcation of the protoxylem appears while the metaxylem retains its central position (fig. 11). At a slightly higher level the bifurcation is broader as the protoxylem is differentiated outward and the metaxylem laterally, so that each bundle now consists of two strands with one strand of each oriented on a clockwise curve and the other on a counter-clockwise curve from the original position. A parenchyma, which is destined to become the pith, appears in the central region (figs. 12, 13). At the level of the cotyledonary plate, the xylem has been oriented so that the curve is approximately  $30^\circ$  from the original position; and at the level where the meristem of the stem is cut off from the cotyledons, the xylem of the bundles has been gradually oriented to the tangential or  $90^\circ$  from its original location (fig. 15). After the tangential stage, the transition occurs in the petioles of the cotyledons. In the lower portion of the petioles, the xylem has been oriented on a curve of from  $90^\circ$  to  $135^\circ$  from the central position, and some distance above this the true endarch condition or  $180^\circ$  from the original position is reached (figs. 16-18).

The primary phloem is also reoriented during transition. In the root and lower hypocotyl it is differentiated in a radial position with respect to the primary xylem; at a higher level of the hypocotyl a bifurcation of the two phloem bundles occurs and the halves of each bundle are gradually oriented on clockwise and counter-clockwise curves until they lie adjacent to the metaxylem, and each phloem bundle appears in a position lateral to the xylem strand nearest it (figs. 11-14). Differentiation along the same curves continues until the phloem is gradually oriented so that each strand lies on the same radius with the xylem strand nearest it, and typical collateral bundles are thus formed (figs. 15-18). These bundles are the median cotyledonary traces.

The cotyledonary plate is distinguished from the hypocotyl by the differentiation in the procambial cells of two groups of two bundles each, lying  $180^\circ$  apart and in radial position to the transition bundles, from which they are separated by a narrow band of parenchyma (fig. 14). The bundles in each group are at first closely



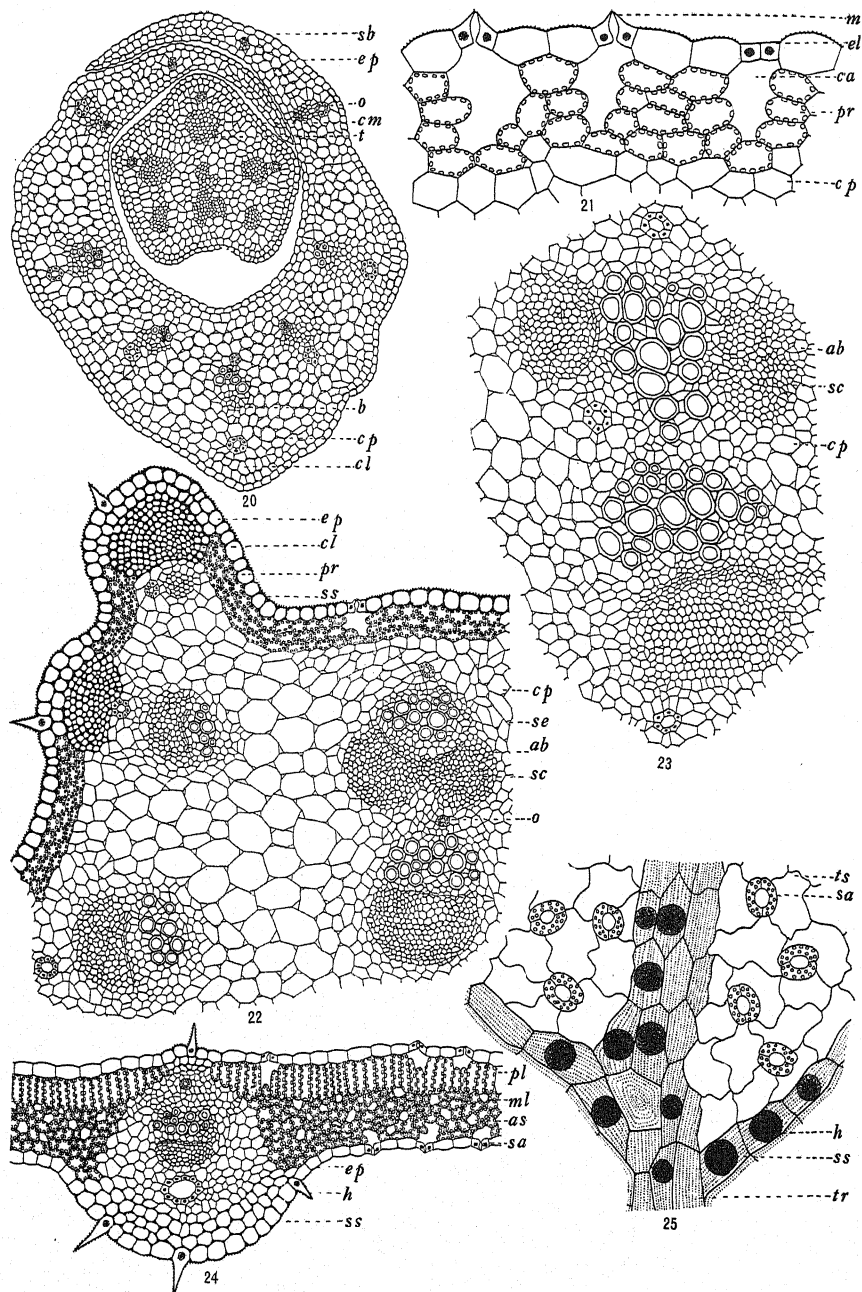
FIGS. 11-19.—Fig. 11, transverse section of lower hypocotyl showing first stage in transition,  $\times 550$ ; fig. 12, cross-section of hypocotyl with metaxylem oriented in a more lateral position,  $\times 550$ ; fig. 13, transverse section of upper hypocotyl showing change in orientation of xylem and differentiation of pith,  $\times 550$ ; fig. 14, cross-section of cotyledonary plate showing reorientation of xylem and phloem and differentiation of lateral cotyledonary bundles,  $\times 220$ ; fig. 15, transverse section showing orientation of transition bundles as cotyledons are being differentiated from central meristem,  $\times 160$ ; fig. 16, cross-section of cotyledons and central meristem with transition bundles tangential,  $\times 220$ ; fig. 17, transverse section of transition bundles in petioles of cotyledons almost endarch, all other tissues omitted,  $\times 440$ ; fig. 18, cross-section of transition bundles in petioles of cotyledons with xylem in endarch position,  $\times 440$ ; fig. 19, transverse section of cotyledonary plate showing orientation of triarch bundles in transition,  $\times 440$  (c, cambium; ep, epidermis; lt, lateral cotyledonary bundle; mt, median cotyledonary bundle; mx, metaxylem; o, oil duct; p, phloem; pa, parenchyma; pc, pericycle; ps, separated xylem arm; pt, pith; px, protoxylem point; t, leaf trace).

associated with each other but are soon separated by a few parenchyma cells which develop between them. All of the parenchyma gradually broadens, and at successively higher levels all bundles are separated by increasingly broader bands of this tissue. As the cotyledons are diverged from the stem, two bundles, one from each group, pass into each cotyledon and become the lateral cotyledonary traces (figs. 15-18). These bundles are developed with endarch arrangement of their elements and take no part in the transition.

#### LEAVES

When the base of a young leaf is diverged from the stem it is roughly crescent-shaped, with overlapping ends, and at this early stage there is no distinction between its tissues except that the bundle meristems consist of cells which are densely protoplasmic and much smaller than those surrounding them. The base increases rapidly in size and at the same time tissues are reoriented and mature. The epidermis is differentiated and collenchyma develops beneath the ridges, being limited by the photosynthetic cortical parenchyma which is centripetal to the grooves and consists of from three to four layers of cells (figs. 20, 23, 27). A continuous zone of cortical parenchyma develops centripetal to these tissues and occupies the center of the petiole, thus surrounding the bundles.

Concomitant with the development of the various tissues is that of the bundles, which are initiated in groups and are gradually separated by the parenchyma which is differentiated between them. At the same time they are gradually reoriented so that eventually they are located at regular intervals around the base, with the xylem facing the ventral and the phloem the dorsal side. At the center from one to three bundles appear, depending upon the size of the base; and on either side of these, small and large bundles alternate with both types, gradually decreasing in magnitude as the ends are approached (fig. 20). As this diminution in size is progressing, the xylem cells, always fewer in number than the phloem, are differentiated less frequently and the bundles which normally are collateral may be reduced to phloem only. Sclerenchyma, in the form of caps, is developed as the bundles mature, with a heavy cap at the xylem and a smaller one at the phloem pole. Oil ducts are associated with all of the bundles but are always separated from them by a few



FIGS. 20-25.—Fig. 20, transverse section of sheathing leaf base and central meristem showing arrangement of bundles,  $\times 110$ ; fig. 21, transverse section of portion of petiole with stomata and stomatal cavities,  $\times 550$ ; fig. 22, cross-section of portion of petiole showing position and anastomosis of bundles,  $\times 220$ ; fig. 23, transverse section of central portion of petiole with several bundles anastomosing,  $\times 220$ ; fig. 24, cross-section of portion of leaf showing small vein and photosynthetic cells,  $\times 220$ ; fig. 25, surface view of leaf with two types of epidermal cells and position of hairs and stomata,  $\times 220$  (*ab*, anastomosing bundles; *as*, air space; *b*, bundle; *ca*, stomatal cavity; *cl*, collenchyma; *cm*, central meristem; *cp*, cortical parenchyma; *el*, section through end of stoma; *ep*, epidermis; *h*, hair; *m*, median section of stoma; *ml*, mesophyll; *o*, oil duct; *pl*, palisade cell; *pr*, photosynthetic parenchyma; *sa*, stoma; *sb*, sheathing leaf base; *sc*, sclerenchyma; *se*, intercellular space; *ss*, striation; *t*, leaf trace; *tr*, tabular cell; *ts*, tortuous cell).

parenchyma cells. In juxtaposition to the central bundle there may be as many as four oil canals; other large bundles are associated with two ducts, a small one near the xylem and a larger one between the phloem and the groove of the collenchyma; while one duct near the phloem appears near small bundles. All of these ducts are schizogenous in origin and develop when the tissue is still in a meristematic stage. Usually four cells which are centered about a common point become densely protoplasmic; and a split, minute at first but gradually increasing in size, separates them at the central point, forming a small canal with the four bounding cells functioning as epithelial cells. As the latter divide the duct is enlarged.

There is a gradual constriction of the leaf base in the acropetal direction. As it merges into the stalk of the petiole, the central portion becomes broader and the ends are restricted, while the groove becomes very shallow and may be eliminated entirely if collenchyma is developed in the adaxial side. In this way the stalk is rounded with a very shallow groove or flattened surface on the ventral side.

The tissues of the stalk are similar to those of the base, except that the photosynthetic parenchyma is broader and contains more plastids (fig. 22). The arrangement of the bundles varies considerably, for as the petiole is constricted there is a reorientation, so that some are located about the periphery with the protoxylem centripetally placed while others appear in the central parenchyma with the xylem directed toward the ventral side. A row of from two to five bundles appears at the middle of the parenchyma and other rows are found on either side of this, varying in number and magnitude according to the size of the petiole. While reorientation is in progress many changes occur in the magnitude and relative position of the tissues of the bundles, for they separate and anastomose frequently. When the change to the stalk is completed the peripheral bundles are relatively stable in size, shape, and position; but those centrally placed continue to separate, reorient, and anastomose throughout the length of the petiole. Since the bundles are differentiated in groups in the base, they tend to separate and then anastomose, thus evolving various types. Those consisting of phloem or sclerenchyma occur frequently, while those consisting of xylem are rare and all three

types may be developed by the separation of small portions of tissue from the main mass. Bicollateral forms are common and are evolved when two bundles of essentially the same magnitude anastomose. The xylem cells unite, which seems to be a common tendency in spite of the fact that a reorientation of  $180^\circ$  is sometimes necessary, while the phloem appears on either side of the xylem and the xylem sclerenchyma caps have become weak and are gradually developed in lateral positions (fig. 23). Amphicribal types are formed by the anastomosis of from three to eight bundles, with the xylem uniting at the center and the phloem groups partially or wholly surrounding them; amphivasal types are rare and are evolved when anastomosed traces pass out of the petiole or midrib into the leaflets. The xylem is oriented about a group of phloem cells and an amphivasal type results. It is possible to confuse compound collateral bundles with simple collaterals, but they can usually be distinguished by the excessive number of associated oil ducts and the slight irregularities in the position of tissues. They are developed when bundles are so oriented that the xylem and all like tissues anastomose laterally (fig. 22).

Anomalous types are exceedingly common and present unusual arrangement of tissues. Two of these are of such frequent occurrence that a brief description seems permissible.

One type is formed during the anastomosis of a compound collateral or bicollateral bundle, when a few of the xylem cells are separated from the main group and are forced out between the phloem cells, so that when anastomosis is completed there are either one or two small groups of xylem extraneous to the phloem and  $90^\circ$  from the main mass. The second type is evolved when the phloem sclerenchyma caps are gradually oriented between the phloem and some of the xylem, and phloem cells are differentiated outwardly, so that the sclerenchyma is surrounded by phloem (fig. 23).

It is impossible to enumerate with any degree of accuracy the number of bundles in any petiole, but a rough estimate may be made from the position of the various tissues and the number of associated oil ducts. The leaf traces increase in number with the magnitude of the petiole, being five in the first leaf and conservatively estimated at fifty in the large leaves.

The meristem of the young leaflet resembles that of the petiole, since there is no clear distinction between the tissues save that the bundle meristem is denser; but growth is rapid and there is a simultaneous development of all elements. The epidermis is similar on the upper and lower surfaces. Beneath it on the upper side, and limited by the tissues connected with the veins, a row of palisade cells is differentiated. These are rounded, much longer than wide, and densely filled with chloroplasts (fig. 24). The mesophyll is composed of relatively small, somewhat angular cells, all containing chloroplasts and interspersed with air passages which are connected with stomatal cavities. The vascular tissue which ramifies through the leaf consists of bundles surrounded by closely packed parenchyma. Sclerenchymatous caps are developed on both poles, and oil ducts are associated as in the petiole. The reorientation and anastomosis of bundles which occurs in the petiole continues throughout the midrib.

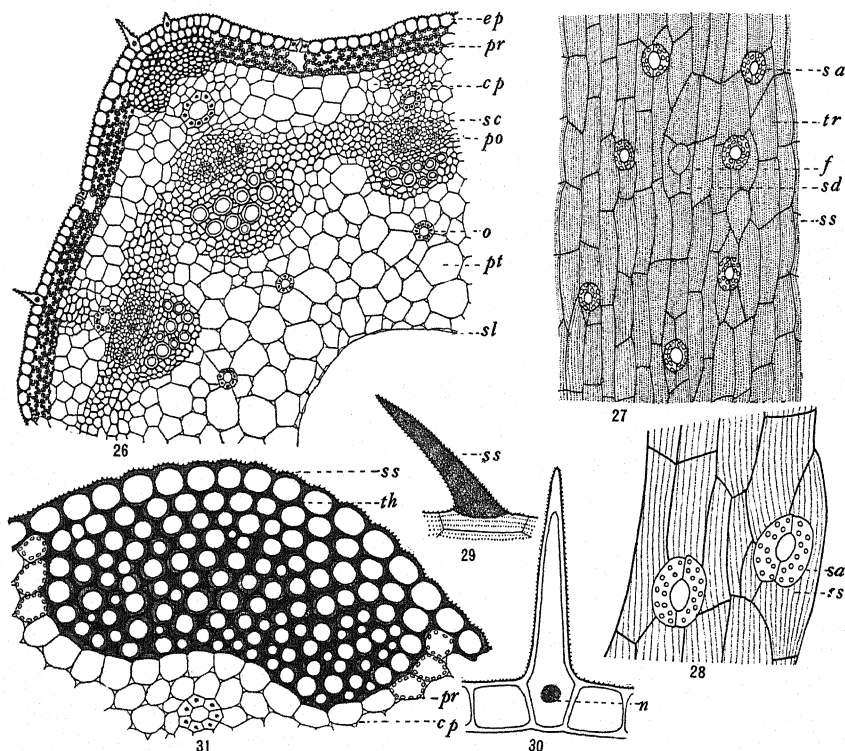
#### FLORAL AXIS

The floral axis is differentiated during the first year but normally exists as a meristem until the spring of the second. The epidermis, photosynthetic cortical parenchyma, and collenchyma differ in no essential features from those of the petiole, since the structure and relative position are similar. The cortical parenchyma, however, is limited centripetally by the bundles, which with the intervening sclerenchyma form a band whose undulations follow the ridges and grooves of the outer surface. Within this band the broad central pith is differentiated. The bundles are similar to those of the petiole, except that oil ducts are always developed within the phloem while in the petiole they could be traced in rare instances only, probably owing to reorientation and anastomosis which are not nearly so marked in the axis. When the bundles are initiated they are discrete, but a meristem which rapidly becomes sclerenchymatous develops between them. Their number and magnitude vary, and large and small bundles alternate, the former being developed centripetal to the ridges and the latter to the grooves.

Oil canals are associated with the bundles, but those near the xylem are differentiated in the pith, and as the axis grows older, the epithelial cells lose their dense protoplasm and appear to be func-



tionless. The oil ducts which are developed in the phloem are usually three in number, and are bounded by four, rarely five, epithelial cells which seem to be incapable of mitosis, resembling the pericyclic canals in this respect. The pith loses its power of cell division early in ontogeny, and as the axis increases in size, the walls of its cells



FIGS. 26-31.—Fig. 26, transverse section of portion of small, mature floral axis showing tissues and schizogen split,  $\times 220$ ; fig. 27, surface view of floral axis showing striations and stomata,  $\times 220$ ; fig. 28, detail of fig. 27 showing twin stomata,  $\times 550$ ; fig. 29, surface view of hair,  $\times 440$ ; fig. 30, section of hair showing internal structure,  $\times 550$ ; fig. 31, transverse section of collenchyma,  $\times 220$  (cp, cortical parenchyma; ep, epidermis; f, initial cell; n, nucleus; o, oil duct; po, phloem oil duct; pr, photosynthetic parenchyma; pt, pith; sa, stoma; sc, sclerenchyma; sd, subsidiary cell; sl, schizogen split; ss, striation; th, collenchymatous thickening; tr, tabular cell).

stretch. Eventually those at the center become weak and are ruptured, and a schizogen split results, leaving the peripheral cells intact (fig. 26). This split occurs in the internodes only, for the

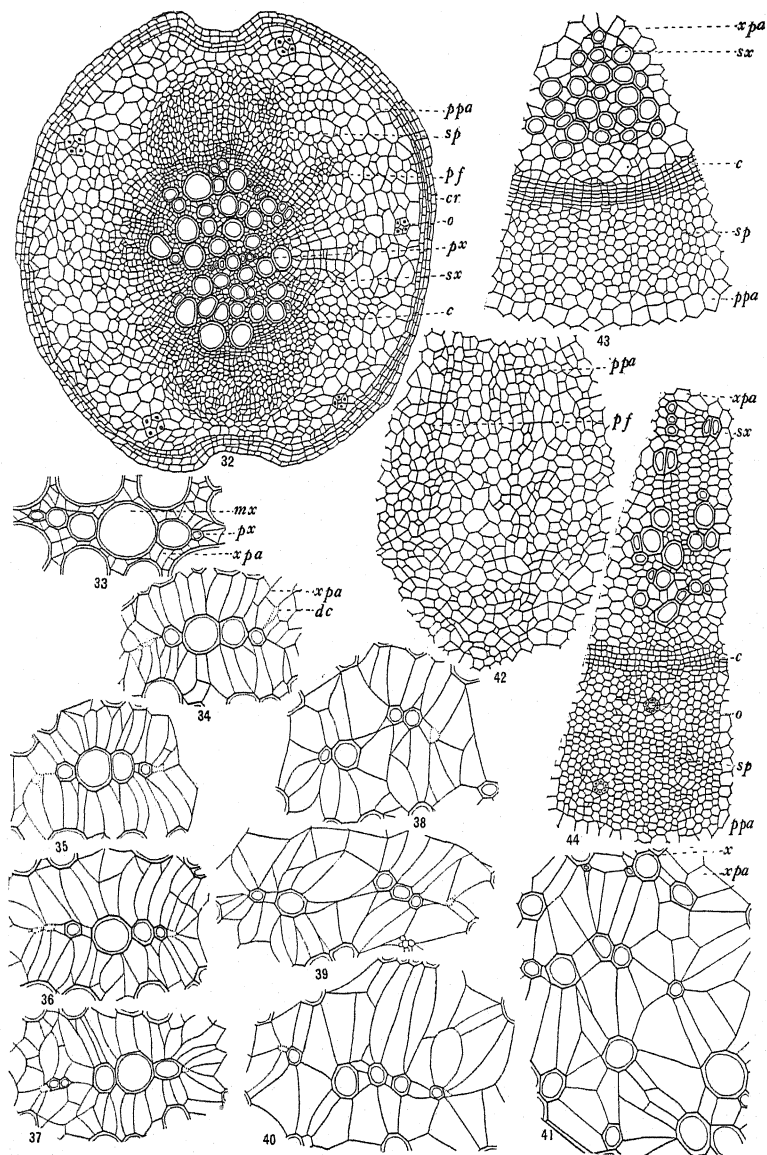
tissue at the nodes is continuous, since it defines the region where the leaf traces depart. Lignification of the floral axis commences as the fruits are maturing, beginning in the acropetal region and proceeding basipetally. The entire vascular band becomes woody and brittle, and this in all probability assists in the distribution of fruits, since the umbels and umblets are broken off soon after the fruits ripen and are carried by the wind in the same manner as are tumble-weeds.

#### SECONDARY GROWTH

In the root and hypocotyl, secondary growth commences with the enlarging of the first leaves, or about seven days after the plant has emerged from the ground, and follows the usual lines. A cambium is differentiated in the procambial strands centripetal to the primary phloem and develops rapidly. There is a lateral extension through the parenchyma which separates the primary phloem from the xylem. In this way the cambial cells are united in the pericycle opposite the protoxylem points, thus forming the cambial ring. Xylem and phloem are laid down in the usual manner but much of this secondary tissue remains in a parenchymatous condition during the first year. In the other organs a cambium is differentiated from the procambial cells in the bundles early in ontogeny.

Secondary thickening in the root and hypocotyl naturally pushes the pericycle, endodermis, cortex, and epidermis outward; and when pressure from within is sufficient, they are stretched and weakened, and since cell division and growth have ceased, they are ruptured and eventually disintegrate. The cortex is ruptured first and carries the epidermis with it. At the same time the endodermis loses its dense protoplasmic content, which causes the Casparian strips to be readily distinguished. The endodermis then sloughs centrifugal to the oil ducts but persists opposite the phloem for a short time, so that the formation of periderm is retarded. When the endodermis finally disintegrates, a shallow groove has been formed (fig. 32).

The pericycle divides rapidly in developing the periderm, and the pericyclic oil ducts are separated by constantly increasing intervening cells, and appear at regular intervals about the periphery just inside the cork. They are readily distinguished because of the limited number of epithelial cells, which gradually lose their protoplasm and dis-



Figs. 32-44.—Fig. 32, transverse section of root showing tissues in early stage of secondary growth after disintegration of epidermis, cortex, and endodermis,  $\times 160$ ; figs. 33-41, disintegration of primary xylem (33, diarch xylem plate with protoxylem intact and parenchyma cells before much increase in size,  $\times 220$ ; 34, xylem parenchyma cells increased in size and protoxylem disintegrating,  $\times 110$ ; 35, parenchyma cells wedging between metaxylem,  $\times 110$ ; 36, disintegration and separation of metaxylem by parenchyma,  $\times 110$ ; 37-39, increase in size of parenchyma cells and separation and disintegration of metaxylem and protoxylem, the latter hardly distinguishable in 38 and 39,  $\times 110$ ; 40, all xylem separated by parenchyma,  $\times 110$ ; 41, transverse section of xylem at center of root in which primary xylem is no longer distinguishable and secondary cells are being separated by parenchyma,  $\times 110$ ); fig. 42, transverse section of secondary phloem showing separation of fibers by parenchyma,  $\times 220$ ; fig. 43, transverse section of young bundle,  $\times 220$ ; fig. 44, transverse section of older bundle,  $\times 220$  (c, cambium; cr, cork; dc, disintegrating cells; mx, metaxylem; o, oil duct; pf, phloem fibers; ppa, phloem parenchyma; px, protoxylem point; sp, secondary phloem; sx, secondary xylem; x, xylem; xpa, xylem parenchyma).

appear. The secondary phloem is largely composed of parenchyma with scattered groups of phloem and fibers. Immediately after disintegration of the outer tissues, the primary and early secondary phloem groups may be seen  $180^{\circ}$  apart and located near the periderm; but the rapid growth of the parenchyma continually forces the cells outward and separates them into strands and later into small groups of cells so that they soon become indistinguishable. The phloem parenchyma is much more abundant than the xylem and all of its cells are filled with minute starch grains. Growth appears to be due not to cell division but to enlargement of the individual cells, and it constitutes the greater portion of the mature root, being from ten to thirty times as large as the xylem (fig. 42).

The secondary xylem is differentiated in the form of uniseriate or biseriate (rarely triseriate) rays with constantly widening intervening bands of parenchyma, two of which are unusually broad and lie on the same diameter with the primary xylem. The vessels of the rays and the fibers are gradually separated by the parenchyma, since the cells of the latter increase greatly in size as the root grows older and the very early secondary xylem loses all semblance to ray arrangement, appearing in transverse section as a relatively large circular area at the center of the root with the vessels scattered through the parenchyma; while that which is differentiated later retains the ray formation with single vessels or in strands of from two to six cells and separated by the parenchyma.

Secondary thickening in the permanent lateral roots is similar to that of the primary, but proliferation of the tissues is not so extensive and the cells remain relatively small while lignification occurs early in ontogeny.

#### DISINTEGRATION OF PRIMARY XYLEM

The primary xylem remains at the center of the stele for a short time after the outer tissues have sloughed, then the parenchyma cells which separate it from the secondary xylem and which were differentiated in a narrow zone between them increase in size, causing a much wider separation. Concomitantly the vasicentric parenchyma cells increase in magnitude and wedge between the cells of the primary xylem. The protoxylem points are the first to be af-

fectured by this pressure, for the walls lose their rigidity and are soon seen in various stages of disintegration (figs. 33-37). They finally disappear, for no trace of them is found in either transverse or longitudinal section and they are apparently resorbed (figs. 38-41). The metaxylem cells are separated into groups and then individual cells, and eventually are scattered in the circular central area where they cannot readily be distinguished from the early secondary vessels (figs. 40-41). In the hypocotyl the pith separates the two archs of the primary xylem plate and the primary and early secondary cells appear scattered in a circular band about it. In the root tip there is little proliferation of parenchyma because lignification begins early and consequently the diarch primary xylem plate remains intact.

### Summary

1. The mature root of the parsnip represents the true primary root together with the hypocotyl. A longitudinal section shows that the major portion is composed of phloem parenchyma whose cells are filled with starch grains. The central portion of the root is occupied by a core of xylem, and in the hypocotyl pith lies within the xylem. Coarse leaves are diverged from the foreshortened stem during the first year and a coarse columnar axis bearing leaves, flowers, and fruit is developed during the second year.

2. The primary tissues of the root consist of xylem differentiated as a diarch and two groups of phloem radially arranged and separated by interstitial parenchyma, also a pericycle, endodermis, cortex, and epidermis. The hypocotyl consists of the same tissues except that pith is differentiated at the center and separates the xylem.

3. Oil ducts are differentiated in the pericycle and primary phloem, and are relatively ephemeral.

4. A very small number of plants appeared in which the hypocotyl seemed to be triarch and in which triple cotyledons were developed, but it was determined that the third arch resulted from the splitting of one of the bundles while in transition; and since the associated roots were diarch in every case and various irregularities in structure were noted in each of these plants, they cannot be considered true triarchs.

5. Permanent and transient secondary roots are initiated in the

pericycle, and other groups of laterals are differentiated in the phellogen near the free conductive tissue of transients which have sloughed off.

6. The foreshortened stem differs little in structure from the hypocotyl, except that the pith is broader and the leaf traces form a band of bundles.

7. The transition commences in the lower part of the hypocotyl, and the bundles have been reoriented to a tangential position (or  $90^\circ$  from the original central position) at the level where the cotyledons diverge from the stem and the reorientation from tangential to true endarch occurs in the petiole of the cotyledons. Lateral cotyledonary traces accompany the transition bundles above the cotyledonary plate.

8. The large coarse leaves vary greatly in texture, color, and the number of leaflets involved. The petiole consists of an epidermis, collenchyma, photosynthetic and cortical parenchyma, and bundles which are normally collateral but which separate, reorient, and anastomose frequently, and in these processes various types of bundles are evolved. The blade is composed of an epidermis whose cells are of two types, tabular and tortuous, also palisade cells and spongy mesophyll.

9. Hairs and striations are developed on the ridges of the petiole and floral axis, and on the veins of leaves. Stomata appear on the stem, in the grooves of the petiole and axis and tortuous cells of the leaves.

10. The floral axis is initiated during the first year and develops rapidly during the second. It consists of an epidermis, collenchyma, photosynthetic and cortical parenchyma, a band of bundles, and a central pith which develops a schizogenous split at the internodes. Flowers and fruit develop during the second year.

11. Secondary thickening involves the activity of a cambium, and in the root and hypocotyl the tissues centripetal to the pericycle are sloughed off soon after secondary growth commences and a very regular periderm is developed by the proliferation of pericyclic cells. In the other organs much of the growth is due to cell enlargement, although in the bundles there is a limited amount of cambial activity.

12. The greater portion of the root and hypocotyl remains in a parenchymatous state during the first year, and increase in size is accomplished through the enlargement of individual cells. The primary xylem cells are forced apart by the growth of the xylem parenchyma and the protoxylem is gradually resorbed, while the metaxylem is scattered through the parenchyma and early secondary xylem, from which it is practically indistinguishable.

CHICAGO, ILLINOIS

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# LEADER, NEEDLE, CAMBIAL, AND ROOT GROWTH OF CERTAIN CONIFERS AND THEIR INTERRELATIONS

RAYMOND KIENHOLZ

(WITH FIVE FIGURES)

## Introduction

The interrelation between the vegetative and reproductive activities of various plants has been investigated extensively of recent years, but relatively little is known regarding the interrelation between the various growth activities and growth regions of perennial plants, particularly forest trees. Most of the work which has been done deals with the activity of a single or at most two growing regions. Leader elongation and cambial growth have been most frequently investigated, but only rarely have measurements of both of these growth activities been at all consistently recorded for the same species or individual under observation. There seems need, therefore, for measurements of the growth of shoots, leaves, cambium, and roots of the same individual or the same species during the same growing season. Research carried out at the Yale Demonstration and Research Forest near Keene in southern New Hampshire over a period of several years gives a fairly clear, coordinated picture of the interrelation between the several growth activities of red pine and white pine.

During the summer of 1931, the writer measured at weekly intervals the growth of the leader, needles, and cambium of red pine, the leader and needles of white pine, and the leader of several other species of conifers. These data will be discussed together with data on root growth supplied by the work of C. L. STEVENS (24).

The growth of the leader has been much investigated, particularly in relation to meteorological conditions. For a discussion of this work reference is made to the recent article by BALDWIN (1) and to résumés by ROMELL, BURGER, and HERTZ cited by BALDWIN. Only the literature dealing with certain specific phases of tree growth will be discussed in this paper.



MATERIALS USED.—The various groups of conifers observed and measured were all located in the Yale Forest. This forest has been described by TOUMEY (27) and need not be discussed here. A group of red and white pines located in one of the plantations was arbitrarily selected as the standard for each species. Other groups of red and white pines of different ages and growing on different sites were measured and compared with the standard. A few other species were included for comparison. The material used is summarized in table I.

TABLE I  
DESCRIPTION OF TREES MEASURED

SPECIES	NUMBER USED	AGE	HEIGHT (FEET)	DIAMETER AT 1 FOOT (INCHES)	SPACING
Red pine.....	12	9	4.9	1.3	4×4
White pine.....	10	10	4.0	0.9	6×6
Pitch pine.....	11	Vol.*	5.8	1.6	Irregular
White spruce.....	20	10	4.0	1.0	6×6
Balsam fir.....	5	Vol.*	15.0	3.7	Irregular

\* Volunteer trees, age unknown.

The soil was in all cases a fine sand, increasing to coarse sand and gravel at lower depths, and with a small admixture of humus in the upper layers. The better sites had a slightly finer, heavier soil with more humus material.

If the curves of leader elongation obtained by C. L. STEVENS (24) in 1928 and 1929, and those obtained by R. D. STEVENS (25) in 1929, are compared with those obtained by the writer, it will be seen that the time of beginning and ending of leader elongation varies but slightly, while the time of most rapid elongation falls within a few days of June 10 in every case. From this it would appear that the course of leader growth varies but little from year to year. Furthermore, climatic conditions which would hasten or delay one of the growth activities would probably have a similar effect on the others, hence the interrelation between the various growth activities would not be greatly altered from year to year.

The species studied were red pine (*Pinus resinosa* Sol.), white

pine (*P. strobus* L.), pitch pine (*P. rigida* Miller), white spruce (*Picea glauca* (Moench) Voss), and balsam fir (*Abies balsamea* (L.) Miller).

## Results

### LEADER ELONGATION

The elongation of the leader was measured at weekly intervals during the growing season by means of a measuring device consisting of a triangular head sliding on a graduated blade which rested on a brass nail driven into the older part of the main axis (12).

RED PINE.—In the standard trees the leader began to elongate late in April, increased rapidly to a maximum rate of 16.1 mm. per day during the week ending June 10, and decreased rapidly and then gradually until growth ceased about August 15, a period of about 104 days (table II, fig. 1). The exact time of beginning and ending of leader elongation is difficult to determine because of the slow and intermittent swelling of the bud in the spring and the formation of the new terminal bud in late summer, which last is not properly elongation of the season's leader. The period of most rapid growth was distinctly marked, however, and extended roughly from May 15 to July 15 (60 days), during which time 96 per cent of the total growth of the season occurred.

Another group of eleven red pines had essentially the same seasonal course of growth as the standard trees. Five red pines, 6 years older than the standard trees, began and ended growth slightly later than the standard trees and reached their maximum growth rate June 17 instead of June 10. The slight difference may be due to the greater sluggishness of the older trees.

A group of seven red pines began and ended their leader elongation sooner than the standards and reached their maximum rate June 2 instead of June 10. The earliness of these trees compared with the standard trees was probably due to the fact that they were located on a southwest slope where air drainage was better and spring air temperature higher than for the checks. The mean air temperature in this group for the first two weeks of May was 54° compared with 52° F. for the standards.

TABLE II  
MEAN DAILY ELONGATION OF LEADER SHOOT (IN MM.)

SPECIES	No. USED	MAY 1	MAY 6	MAY 13	MAY 20	MAY 27	JUNE 3	JUNE 10	JUNE 17	JUNE 24	JULY 1	JULY 8	JULY 15	JULY 22	AUG. 5	AUG. 18
Red pine.....	12	.....	1.0	1.7	3.5	5.7	15.1	16.1	13.1	9.2	6.7	1.4	1.0	0.9	.....	0.1
White pine.....	10	.....	.....	1.2	2.2	3.6	7.2	10.0	8.6	6.4	6.0	4.8	2.5	0.6	0.1	0.1
Pitch pine.....	11	.....	.....	1.2	2.4	5.0	12.6	14.7	12.3	5.1	2.3	0.5	1.5	0.4	0.4	.....
White spruce.....	20	.....	.....	.....	1.2	2.8	7.8	10.7	7.5	7.1	5.0	1.9	0.2	0	0	.....
Balsam fir.....	5	.....	.....	.....	0	2.4	4.2	5.5	6.6	6.8	5.2	3.4	2.4	0	0	.....

WHITE PINE.—Leader elongation in the white pine standard trees started slightly later than in the red pines, but reached its maximum rate at the same time (June 10) and ceased growth at the same time. The maximum growth rate was 10.0 mm. per day compared with 16.1 mm. for red pine. Most of the growth (96 per cent) occurred, as in red pine, from May 15 to July 15.

A group of five older white pines began and ended growth later but reached their maximum rate at the same time as the standards. This behavior is somewhat similar to that of the older red pines.

A group of four white pines located on a southwest facing slope had a seasonal course of elongation similar to that of the red pine grown under similar conditions; that is, they began and ended growth earlier than the standard trees and reached their maximum June 2 instead of June 10.

PITCH PINE.—Leader elongation began about the same time as in white pine, increased rapidly to a maximum of 14.7 mm. per day (June 10), and fell off rapidly by early July. An increased elongation occurred July 15 owing to the formation of a very long bud characteristic of this species. It had completed 97 per cent of its total growth for the season from May 15 to July 15 (60 days).

WHITE SPRUCE.—Leader elongation had barely started by May 13, but

increased rapidly to a maximum rate of 10.7 mm. per day the week ending June 10, and ceased growth July 23. It was possible to determine more accurately when leader elongation began and ended in spruce than in red and white pine. Practically the entire leader elongation took place from May 15 to June 15.

**BALSAM FIR.**—No elongation occurred before May 20, but by May 27 the leader was growing rapidly. Leader growth increased to

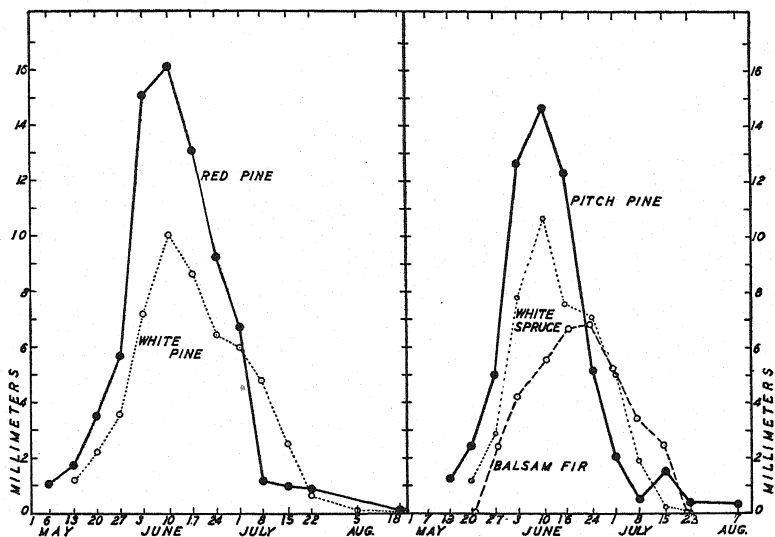


FIG. 1.—Seasonal course of leader elongation in several species of conifers (daily increment by weekly periods in mm.).

a maximum rate of 6.8 mm. per day on June 23, two weeks later than in the other species. It ceased July 23. Balsam fir has a short growing season, starting later and ending earlier than the other species studied. It practically completed its growth in 65 days as compared with about 105 days for red, white, and pitch pine and 70 days for white spruce.

With the exception of balsam fir, all the species studied reached their peak June 10. Red pine started growth earliest, followed closely in order by white pine, pitch pine, white spruce, and balsam fir, with little difference between the first three species. Growth ceased about the same time in the three species of pines. Spruce and fir

ceased growth slightly earlier than the pines. Balsam fir had a very abrupt beginning and ending of leader elongation; spruce less so; while the pines began and ended growth gradually, probably owing to the way in which their terminal buds swelled in the spring and formed in the later summer.

#### DAY AND NIGHT ELONGATION OF THE LEADER

For a period of 17 days (June 13-29) the leader elongation of the twelve check red pines was recorded every morning and evening at 6:30. This divided the day into halves corresponding rather closely to the periods of daylight and dark and their corresponding temperature changes. Within a few feet of the trees the temperature of the air was recorded by a hygrothermograph and by standard Weather Bureau maximum and minimum thermometers installed in louvered shelters.

With the exception of two days, June 24 and 25, the amount of growth occurring during the night was greater than that occurring during the day (fig. 2). The average increment for the 17 days was night growth 5.93 mm. and day growth 4.47 mm., or in the ratio of 100 to 75. ILLICK (10) reports a similar increase of night growth over day growth for a number of trees; for white pine the ratio was 100 to 70.

When the minimum and maximum temperatures were compared with the amounts of day and night growth, a fair degree of correlation was found to exist between night growth and minimum temperature ( $+0.744 \pm .075$ ) and between night growth and mean temperature ( $+0.747 \pm .075$ ). On cold nights when the temperature dropped to  $42^{\circ}$  or lower, the amount of growth was very slight while on warm nights the growth was greater. During the two times when day growth surpassed night growth the minimum temperatures were low, being  $42^{\circ}$  and  $34^{\circ}$  respectively. The correlation between night growth and maximum temperature of the day before was not statistically significant. There was likewise no significant correlation between day growth and temperature. Saturation deficit was probably more important than temperature in determining the amount of growth per day (20), but no data are available on this point.

The data on night growth show the dependence of growth on temperature, and particularly the retarding effect of cold nights. It seems likely that early in the season (because of colder nights) the amount of growth at night would be less than during the day, as has been shown by TOLSKY (26) for southern Russia.

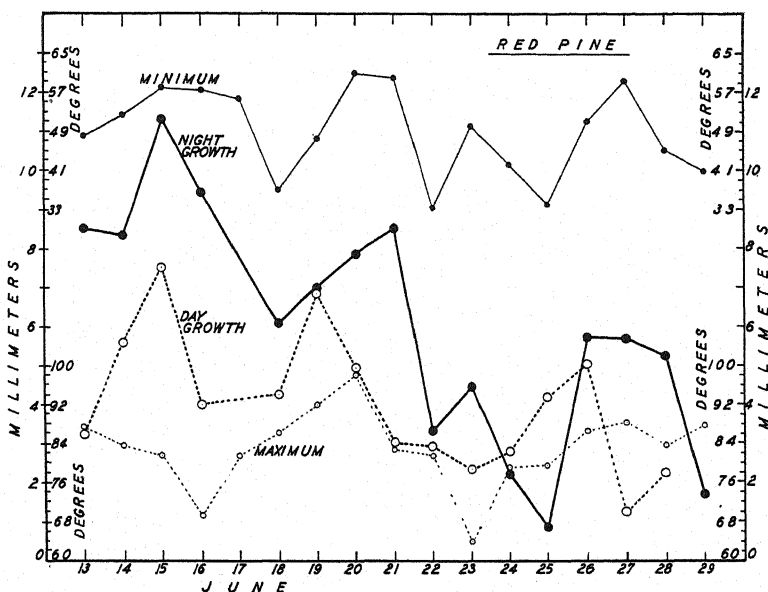


FIG. 2.—Day and night elongation of the leader of red pine in relation to temperature (increment in mm.).

#### NEEDLE ELONGATION

Four needles on the south side and four needles on the north side of each leader were measured at weekly intervals during the growing season. These needles were located on the base, lower middle, upper middle, and tip portions of the leader. Each fascicle in pine is subtended by a bract, the lower part of which is firm and persistent and the upper part of which is chaffy and deciduous. The persistent base of the bract served as an unvarying zero point from which to measure the growth of the needles, particularly when young. When older the celluloid millimeter ruler was slipped between the needle and the twig. In the case of red pine the two needles of the fascicle were

usually the same length, while in white pine the five needles of the fascicle varied somewhat. Their average length was measured in each case. The eight chosen fascicles were marked at their bases with black India ink and the same needles were remeasured each time unless injured, in which case they were discarded.

In order not to injure the delicate underlying needle primordia nor to retard needles or leader growth by too early exposure to drying, the chaffy scales were removed by hand after the needles had already put on some growth. This was particularly true of the basal needles, hence their curve of growth is incomplete. The needles at the tip of the leader, however, were not yet visible above the base when observations began, hence data covering their entire growth period were obtained. There was practically no difference between the growth of the needles on the north and south sides of the leader, hence they were averaged together.

When the growth of the basal needles was compared with that of the needles located higher on the leader a distinct progression was noticed. That is, in the case of the standard red pines the basal needles were 9.4 mm. long on June 1, were growing vigorously on June 12, had reached their maximum growth rate on June 24, and decreased gradually to August 19 (fig. 3). The tip needles on the other hand were not yet visible above the bract base on June 1, but had begun growth by June 12. They reached their maximum growth rate on July 8, two weeks later than the basal needles, and then fell off gradually to cease growth by September 2. The lower middle and upper middle needles followed courses of growth intermediate between those of the basal and tip needles. A wave of growth progresses from the base of the leader to its tip, which causes each needle to pass through its grand period of growth earlier than those above it. Although this progression is marked in the time of starting and the time of reaching the maximum, it is not so marked in the time of ending growth (fig. 3).

In each of the 65 red, white, and pitch pines investigated, the growth of the needles showed the same progression of development from the base to the tip of the leader already described for red pine. However, the difference between the base and tip needles was not so great in white and pitch pine as it was in red pine.

More accurately to indicate the seasonal course of growth of the needles over the entire leader, the eight needles from each tree were averaged together for the trees of the group, thus reducing needle growth to a single curve.

RED PINE.—Because of the danger of damage to the delicate meristematic tissues at the base of the needles the early growth of the basal needles was not obtained, hence the total growing period of the

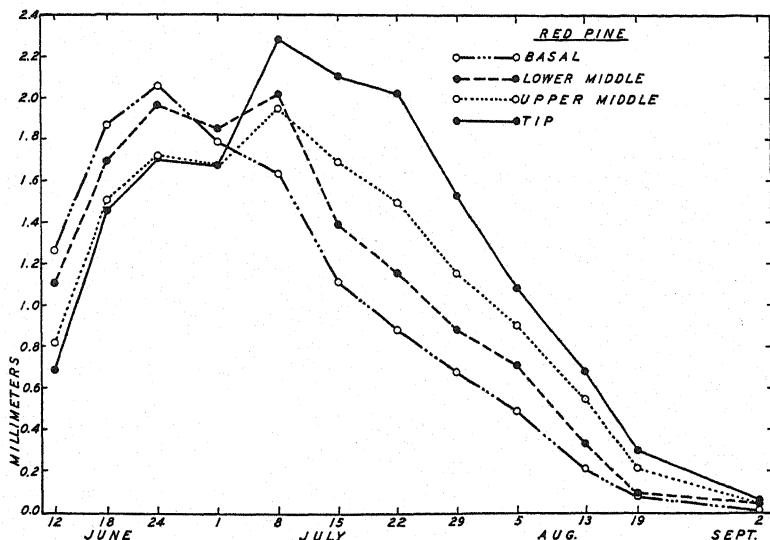


FIG. 3.—Seasonal course of elongation of red pine needles located at different levels on the leader (daily increment by weekly periods in mm.).

needles is only approximately known. But by knowing the length of the basal needles at the time of first measurement (June 1), it can be determined that they started growth about May 15. The tip needles were not showing above the bract base on June 1, but averaged 9 mm. long on June 12; hence they must have started growth shortly after June 1. They grew until September 2, a growing period of 94 days. The basal needles grew from about May 15 to early September, so that the entire time during which needle elongation occurred was about 110 days (table III, fig. 4). Most of the growth (94 per cent) occurred between June 10 and August 10 (60 days).

A comparison of needle elongation showed little difference be-



TABLE III  
MEAN DAILY ELONGATION OF NEEDLES (IN MM.)

SPECIES	No. OF NEEDLES	INITIAL LENGTH, JUNE 2	JUNE 12	JUNE 18	JUNE 24	JULY 1	JULY 8	JULY 15	JULY 22	JULY 29	AUG. 5	AUG. 13	AUG. 19	SEPT. 2
			JUNE 2	JUNE 12	JUNE 18	JUNE 24	JULY 1	JULY 8	JULY 15	JULY 22	JULY 29	AUG. 5	AUG. 13	AUG. 19
Red pine.....	268	3.4	1.03	1.03	1.86	1.74	1.96	1.54	1.39	1.06	0.80	0.44	0.17	0.03
White pine.....	144	4.2	0.55	0.90	0.98	1.08	1.32	1.34	1.10	0.85	0.54	0.17	0.08	0.02
Pitch pine.....	72	4.2	0.80	1.12	1.42	1.24	.....	1.34	0.94	0.70	0.53	0.34	0.30	0.04

tween various groups of red pine trees. There was a tendency, however, for the older trees to start and end growth later than those trees located on the southwest slope, a behavior similar to that noted for leader elongation in these same groups.

WHITE PINE.—Needle elongation started at about the same time as in red pine but ceased slightly earlier. The maximum growth rate was reached about a week later than in red pine. There was very little difference between the different groups of white pine.

PITCH PINE.—The seasonal course of needle elongation was similar in pitch pine and red pine, although the amount of growth was less in pitch pine than in red pine.

An examination of needle elongation showed that nearly every needle measured decreased its rate of elongation during the week ending June 30 as compared with the weeks before and after. Because of this lessened growth rate the curve of needle elongation, particularly in red pine, showed a dip on June 30 with a rise on either side (in other words, a double maximum). Such a curve is unusual, particularly with the two maxima only two weeks apart. The peculiar shape of the curve was not due to a small number of samples since the same dip in the curve was obtained by plotting the mean of more than 250 needles. The fact that nearly all the needles were retarded in their growth rate pointed to meteorological

logical conditions as the cause. The air temperatures at a station near the standard red pine trees were as follows:

	JUNE 24	JUNE 30	JULY 8	JULY 16
Mean maximum.....	82.4	87.3	86.4	85.6
Mean minimum.....	49.0	45.8	57.2	57.2

The week ending June 30 had the highest maximum air temperature, including one day of  $100^{\circ}$  and the lowest minimum including one night of  $34^{\circ}$  F. Moreover, the relative humidity was much lower

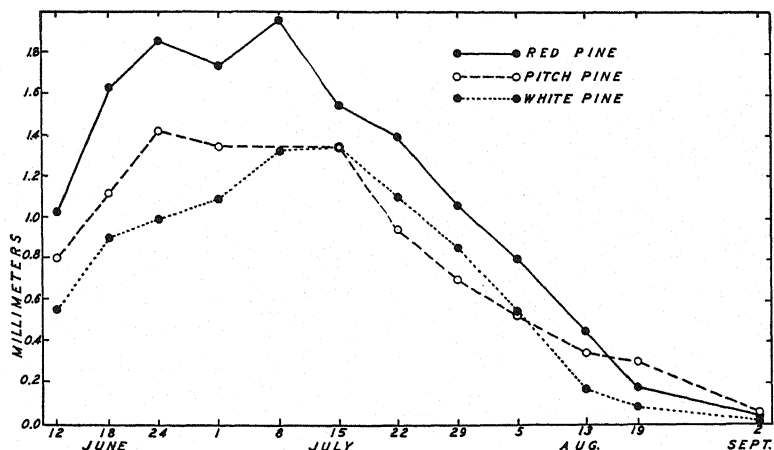


FIG. 4.—Seasonal course of needle elongation in several species of conifers (daily increment by weekly periods in mm.).

during the week ending June 30 than during the following weeks. Meteorological conditions, particularly the cold nights, retarded growth and converted what would probably have been a maximum growth rate into one slightly lower than that of the preceding and succeeding weeks.

#### GROWING REGION OF NEEDLES

In late July a number of needles of red, white, and pitch pine were marked into 2 mm. intervals by means of India ink lines and later remeasured at intervals. In every case all elongation took place at the extreme base of the needle within the protective basal sheath; that portion of the needle above the sheath showed no signs of further elongation. In white pine, the distinct scales of which the

sheath is composed also elongated at their bases. BÜSGEN (3) states that after the first rapid elongation of the young needles, growth proceeds from an active zone at the base.

#### DO THE NEEDLES GROW FOR MORE THAN ONE YEAR?

Considerable controversy has arisen in the past over whether or not the needles of pine grow in length after the first year. KRAUS (14) maintained that they did, while MEISSNER (19) insisted that they did not. HONDA (7), working with *Pinus longifolia* in Japan, found the needles did not grow in length the second year. More recently HAASIS (5) reported elongation after the first year in the needles of Monterey pine defoliated in March, while LODEWICK (16) reported increased length during their second year for the needles of longleaf pine in Florida.

A great number of needles measured in September, 1930, were re-measured in May and October, 1931. These needles were from both red and white pine trees of different ages, growing in the open or in the shade, and from trees defoliated or not defoliated. In no case was there any elongation the second year. Apparently elongation the second year is a matter of species difference and geographical location, with such factors as defoliation also of some importance.

#### DAILY NEEDLE ELONGATION

Four needles on each of the twelve standard red pine trees were measured daily at about 8 A.M. for a period of 24 days (June 30 to July 23) and the mean daily growth rate determined. A comparison of the rate of elongation for each day with the mean temperature for the same period showed a positive correlation of  $+0.627 \pm 0.08$ . This correlation was particularly evident when growth was very rapid or very slow. The maximum temperature showed a correlation of  $+0.618 \pm 0.08$  with the growth rate, while the minimum temperature showed no significant correlation.

#### GROWTH OF CAMBIUM

Dendrographs of the type devised by MACDOUGAL (18) were attached to two red pine trees at about 1 foot above the ground. One of these trees grew in an open 8×8 plantation and was 18 feet high and 6.1 inches in diameter at a height of 1 foot. The other tree grew

near by, partly under a canopy of gray birch and poplar. It was but slightly affected by this competition, however, since it was 16 feet high and 4 inches in diameter at a height of 1 foot. The two records were almost identical, hence their average is here presented.

No growth had taken place by May 5, but by May 12 growth had begun and increased rapidly to a first maximum on June 2, decreasing slightly to increase to a second maximum on July 9. Growth gradually became less and less, ceasing about October 1, a growing period of 150 days. The records of both trees agreed in having their maxima on June 2 and July 9. There was no correlation between the maximum, minimum, or mean air temperatures and the average weekly growth rate, nor between the relative humidity and the growth rate; hence it would seem that the double maximum was not due to climatic conditions but to some internal factor.

The literature on diameter growth of trees shows a number of instances recorded by BROWN (2), FRIEDRICH (4), JOST (11), and LODIEWICK (15) in which there was a flattening of the curve of growth, presumably at the time of transition between spring wood and summer wood formation. Although no histological examination of the tissues was made in this study, the similarity between the results here recorded and those based on periodic microscopic examination of the cambial region (15) points strongly to the view that the first maximum (June 2) was due to spring wood formation and the second maximum (July 9) to summer wood formation.

#### ELONGATION OF ROOTS

No data are available on the root growth of red pine, hence the following data are taken from the work of STEVENS (24, table VII and plate VI) on white pine in 1929. Elongation of the roots of white pine began about the middle of April, reached a first maximum June 8, dropped off to a low growth rate August 3, rose again to a second higher maximum on October 4, and fell off to cease growth in early November, a growing period of about 205 days (fig. 5).

#### INTERRELATION OF GROWTH ACTIVITIES

When leader elongation, needle elongation, cambial growth, and root elongation are compared with one another (fig. 5), an interrelation can be seen between the various growth activities and growing

regions of the tree. Since each growing region draws water, mineral nutrients, and elaborated foods to itself, there must be a constant and probably severe competition for these growth materials which are usually limited in amount. As one growing region increases in activity the attraction it exerts on available growth materials becomes greater (3). This decreases the supply available to some other growing region, which may gradually cease its activities through lack of materials (21). What normally causes beginning and cessa-

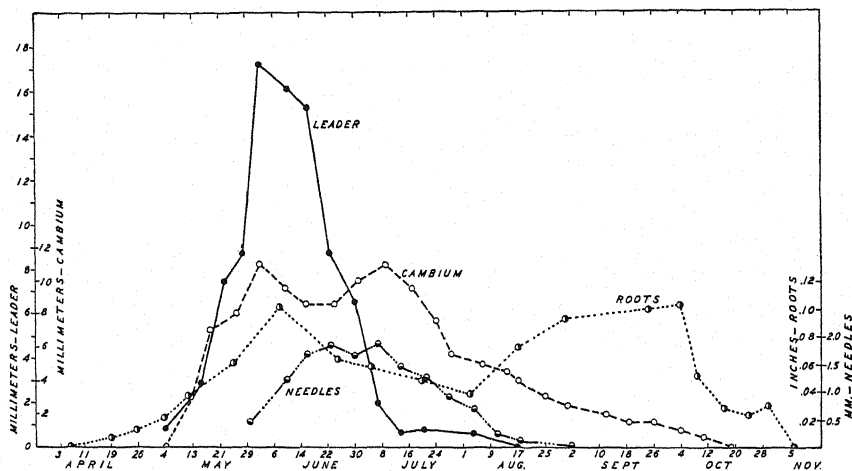


FIG. 5.—Interrelation of the different growth activities of pine (cambium growth expressed as amount of increase in diameter since previous week's reading; all others, daily increment by weekly periods).

tion of activities in the various growing regions of the tree is not known. In the case of leader elongation at least, it was certainly not low temperature since growth ceased in July or August while the temperatures were still relatively high. Nor is it known what causes the double maximum in the rates of cambium growth and root elongation. It seems probable, however, that the cause is to be sought in an internal balance between the different growth activities of the tree, or, as PRIESTLEY (23) states, "the fluctuation in the internal factors correlating growth, including a fluctuating balance of metabolic activities." And when it is realized that the attraction of a growing region may be primarily for water, or various mineral nutrients,

or different elaborated foods at different times, the process becomes even more complex. The present data and discussion can do little more than throw into relief the various problems arising from the interrelation of all growth activities by presenting what is known concerning a species during a growing season.

The first organ to begin growth was the root, followed by the leader and the cambium, and considerably later by the needles. Although there was a slight amount of early root and leader elongation previously, their rapid growth did not begin until about May 1. After May 1 there was a great surge of growth in the leader, in the roots, and in the cambium at approximately the same time. This extensive activity was probably due to the ready availability of a relatively large amount of stored food and the presence in the wood of an abundance of water. Leader elongation reached a maximum (in those trees to which the dendrographs were attached) at the same time as the first maximum of cambium growth (June 2), followed closely by the first maximum of root elongation (June 8). There is probably a causal relation between these three growth activities of the tree connected with the absorption of water by the elongating roots, its transportation by the newly formed spring wood, and its utilization by the rapidly elongating shoots. The slump in growth rate of the leader and roots and cambium appears to be associated with the depletion of reserve foods stored in the tree.

Needle elongation began while the roots, cambium, and leader were growing rapidly and reached its maximum growth rate when roots, cambium, and leader were decreasing their growth rate. The considerable mass of material needed to develop the current set of needles may well have decreased the food supply to the other growing regions of the tree, causing them to grow more slowly. This would be the case whether the food available were from stored material or from food synthesized by the needles. It is likely that most of the food available at this stage of the development was newly synthesized by the old needles, and would thus be readily available to the newly developing current set of needles, since nearness to the supply often determines which organ receives the greater amount of food (17). As these needles become more active photosynthetically through greater surface, they would increasingly make food avail-

able for the second surge of growth of the cambium (July 9). This growth, at first largely cell extension, would be followed by differentiation and maturation of the thick walled, late summer wood cells requiring large amounts of carbohydrates. Late wood cell differentiation might well occur in early August, since BROWN (2) states that late wood formation in white pine begins during the first half of August and is associated with a decrease in growth intensity. Thus the greatest utilization of food by the cambium would occur when most of the other growing regions of the tree were growing relatively slowly, and might account for the decreasing growth rate of the needles and roots.

The behavior of the roots with their greatly reduced elongation during early August is most striking. This decrease in rate of elongation of the roots may be due to the diversion of foods to the differentiating late wood cells as already suggested, or it may be due to the balance between radial growth and elongation in the root itself. Few data are available as to just when radial growth takes place in the roots, but HARTIG (6) believes that it starts late in the season (August). PETERSEN (22) maintains that the midsummer period of decreased root elongation is correlated with maximum radial growth in the older roots.

With a full complement of needles functioning during August and September, food is being produced in abundance, which may result in carbohydrate accumulation. Some of this food is transported to the roots and probably accounts for the increased elongation of the roots in early October, since HOOKER (8) states that roots use carbohydrates; hence an accumulation of carbohydrates in the aerial parts of the plant, which may cause them to cease growth, tends rather to favor root growth. What causes cambial development to cease is not known, although by late September temperature may be a limiting factor. PRIESTLEY (23) suggests that the replacement of sap by air in the tracheids near the cambium may bring about a deficiency of water and cause the cambium to cease functioning. HOWARD (9) suggests that an accumulation of carbohydrates which checks enzyme action brings about the rest period. Root elongation is probably stopped by low temperature.

Thus aside from the first surge of growth during which the leader

reaches a maximum and the roots and cambium reach a first maximum growth rate at nearly the same time, the growth activities of the tree reach their maxima at different times in the order: needles, cambium, and roots. After the store of reserve food has been largely depleted, the interrelation between the various growth activities of the tree seems to be dependent upon the availability of food, water, and possibly mineral nutrients.

A similar correlation between the various growing organs of the cotton plant is suggested by the work of BALLS and HOLTON as analyzed and presented graphically by PEARSALL (21). In cotton, growth of the stem apex was decreased by the growth of large numbers of flowers whose growth was in turn decreased by growth of the fruit. PEARSALL (21) shows a falling off in the rate of elongation of the main root of peas and beans at the time of secondary root production, and increased growth following the removal of the shoot. KNY (13) and TOWNSEND (28) note an increase in the rate of growth in roots following removal of shoots. Such results must always be considered in the light of the amount and the place of storage of food available for growth.

### Summary

1. The seasonal course of leader elongation, needle elongation, and cambial growth of certain conifers, particularly red pine and white pine, was measured during the 1931 growing season near Keene, New Hampshire.

2. Leader elongation in red pine began in late April, reached its maximum by June 10, and ceased about the middle of August, a growing period of approximately 105 days. Ninety-six per cent of this growth was made during the 60 day period from May 15 to July 15. Older trees were slightly later in their seasonal course of leader elongation than were younger trees. Trees located on southwest slopes started growth earlier than those located on level areas.

3. Leader elongation in white pine started slightly later than in red pine but reached its maximum at the same time (June 10) and ceased growth at the same time. Ninety-six per cent of the growth occurred from May 15 to July 15.

4. Leader elongation in pitch pine followed a seasonal course



similar to that of white pine. Ninety-seven per cent of this growth occurred from May 15 to July 15.

5. Leader elongation in white spruce started the middle of May, reached a maximum June 10, and ceased July 23, a growing period of about 70 days.

6. Leader elongation in balsam fir started late in May, reached a maximum June 23 (two weeks later than the other species), and ceased July 23, a growing period of about 65 days.

7. From June 13 to June 29, 60 per cent of the leader elongation of red pine occurred at night, 40 per cent during the day. A correlation of  $+0.744 \pm 0.075$  existed between night growth and minimum temperature. No significant correlation existed between day growth and temperature.

8. The needles located at the base of the leader in red, white, or pitch pine pass through their grand period of growth earlier than do those located higher up on the leader. Those at the tip began elongation about June 5.

9. Needle elongation in red pine began about May 15, reached a maximum late in June, and ceased in early September, a growing period of 110 days. Ninety-four per cent of the growth occurred during the 60 days from June 10 to August 10.

10. Needle elongation in pitch pine was similar to that of red pine; white pine was slightly more tardy in its development than red pine.

11. Needles of the pines investigated grow from a meristematic region at their base. They do not elongate after the first growing season.

12. Daily needle elongation in red pine showed a correlation of  $+0.627 \pm 0.08$  with the mean temperature.

13. Cambial growth in red pine as measured by the MACDOUGAL dendrograph began in early May, reached a first maximum June 2, decreased slightly and reached a second maximum July 9. Growth ceased in early October, a growing period of 150 days. There was no correlation between the meteorological conditions and the double maximum in the growth rate. The first maximum is probably associated with the development of spring wood and the second maximum with the development of summer wood.

14. STEVENS reports that the roots of white pine begin to elongate the middle of April, reach a first maximum June 8, decrease, rise to a second maximum on October 4, and cease in early November, a growing period of about 205 days.

15. There is a certain interrelation between the activity of the different growing regions of the tree. In early June there is a great surge of growth in leader, roots, and cambium at approximately the same time. This spurt of growth is made possible by the great amount of readily available reserve foods stored in the tree. There is probably a causal relation between these different growth activities having to do with the absorption, transportation, and utilization of water.

16. After this first surge of growth the needles reach their maximum growth rate, followed by the second maximum of cambial growth. After nearly all growth is completed and the full complement of needles is functioning, the roots reach their second maximum rate of elongation.

17. It is suggested that there is an internal balance between the metabolic activities of the different growing regions of the plant which determines the time of maximum development of needles, cambium (late wood formation), and roots. After the reserve foods are exhausted this balance is necessitated by limited supplies of elaborated foods, water, and possibly mineral nutrients.

AGRICULTURAL EXPERIMENT STATION  
NEW HAVEN, CONNECTICUT

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# EMBRYOGENY OF CARYA AND JUGLANS, A COMPARATIVE STUDY<sup>1</sup>

LADEMA M. LANGDON

(WITH PLATE I AND FIFTY-SIX FIGURES)

## Introduction

While numerous studies have been reported (HANSTEIN, 1870, WESTERMEIER, 1876, HEGELMAIER, 1878, 1897, FAMINTZIN, 1879, GUIGNARD, 1881, COULTER and CHAMBERLAIN, 1903, SCHAFFNER, 1906, SOUÈGES, 1916-1928, and others) dealing with the embryogeny of angiosperms (including representatives of a large number of families of both Dicotyledoneae and Monocotyledoneae), information relating to the embryological development of the woody ament-bearing angiosperms, especially the Juglandaceae and Fagaceae, remains markedly deficient.

BENSON's contributions (2) were concerned chiefly with pollination and embryo sac phenomena in certain genera of the Betulaceae and Fagaceae. They added little to the true embryogeny of the Amentiferae. CONRAD (7) contributed a brief description of the development of ovule, archesporial tissue, embryo sac, and endosperm in *Quercus velutina*. Seven lines at the close of his paper are devoted to the embryo.

BENSON and WELSFORD (3) described the morphology of the ovule and female flowers of *Juglans regia* and allied genera, with special reference to their vascular structure. Prior to that, the development of the female flowers and pollination in different species of *Juglans* had been dealt with by NAWASCHIN (12, 13), KARSTEN (8), and NICOLOFF (15). In a comparative study of pollen tube features and the male gametophytes of chalazogamens, NAWASCHIN and FINN (14) have also given considerable attention to the embryo sac and fertilization in *Juglans nigra* and *J. regia*; and on the basis of gametophyte characters determined in the *Juglans* type are inclined to regard the

<sup>1</sup> A section of this report was presented before the General Section of the Botanical Society of America at Atlantic City, December, 1932. The investigation has been aided by grants from the Committee on Grants-in-Aid, National Research Council.

Juglandaceae as survivors of a series of extinct transition forms between the two great seed-plant groups. In this connection, they place special emphasis upon a certain distinctive character of *Juglans*, foreign to other angiosperms, namely, the retention here of the "two kernalled generative cell" or the capsular inclosure of the pair of discharged sperm nuclei.

Of interest, particularly to the horticulturist, are the more recent investigations of STUCKEY (19), WOODROOF (20, 21, 22), ADRIANCE (1), and SHUHART (16). STUCKEY has studied certain phases in the development of the pecan flowers, recording for 25 varieties of *Hicoria pecan* data relative to the time of maturity of the pollen and period of receptivity of the pistillate flowers. Fruit bud differentiation and development of the flowers, embryo sac, and fruits of the pecan, with brief reference to embryological characters, appear in a series of papers by J. G. and NAOMI WOODROOF (20-22). Their observations concerning the embryogeny of this form may be summarized as follows: The first division of the egg (occurring eight to nine weeks after pollination) is horizontal, forming a chalazal cell and a large one-celled suspensor, the chalazal cell continuing division in such a manner as to produce a round globular embryo attached at the micropylar end of the sac. A 2- to 4-celled embryo is reported ten weeks after pollination, and a 32- to 64-celled embryo eleven to twelve weeks after pollination, with development of the cotyledons within one month of the first division of the egg.

Recently SHUHART (16) has made a study of the morphology and anatomy of the fruit of the pecan, giving particular attention to the origin and development of the carpellate inflorescence, differentiation of the floral organs, and the vascular organization of the young fruit. The development of the embryo is treated only briefly, his observations confirming those previously reported by WOODROOF and WOODROOF, except that SHUHART finds the orientation of the cotyledons transverse to the plane of the middle septum of the fruit, as described by DE CANDOLLE (6) and NICOLOFF (15).

#### Investigation

Representatives of the Juglandaceae and Fagaceae selected for the studies in progress are *Carya glabra*, *Juglans mandshurica*, *Quercus*

*rubra*, *Q. alba*, and *Fagus ferruginea*. Pollination and fertilization, with derivation and development of the endosperm, are to be investigated in these forms, but particular attention is given to their embryogeny, especially in its later phases, including details of origin of cotyledons, primary root, and plumular bud, with the morphology of the growing points of root, shoot axis, and foliar organs; that is, an attempt is made to bridge the gap between early embryogeny and early seedling development.

The present report presents, in a comparative form, certain of the observations and conclusions reached in the course of the study, particularly as they relate to the Juglandaceae. For this section of the project, collections of material for imbedding were taken at frequent intervals, from April to August, 1931 and 1932, from trees on the campus and in the arboretum of Johns Hopkins University, and from the gardens of Dr. E. A. ANDREWS at Govans, Maryland. Both in *Carya* and in *Juglans*, samples were secured from swelling buds and tips of growing shoots before the appearance of the carpellate flowers. Following the appearance of the carpellate flowers, early in May, collections were made three to four times weekly to the time of pollination (May 6–10 in *Juglans*, May 12–15 in *Carya*), after pollination daily for a period of two weeks; and then at intervals of two to three days through June, July, and early August (periods of embryo and fruit development).

Approximately 350 fixations of *Carya* and *Juglans* were prepared and imbedded through the two collecting seasons. Fixation of the flowers and fruits has been with formalin-acetic-alcohol or with Bouin's fluid, the latter giving greater satisfaction in fixation of young embryo sacs. To insure the best possible penetration of fixing, clearing, and imbedding agents, the cupule and ovarian wall (exocarp) were trimmed from opposite sides of the ovulate flowers and fruits, either in the plane of or at right angles to the plane of the middle septum. In preparing the older fruits for paraffin imbedding, it was found advisable, after exhausting as much air as possible from the tissues, to substitute in dehydration mixtures of ethyl and n-butyl alcohols for the usual ethyl alcohol-xylene series (23). Serial sections, 8–10  $\mu$  in thickness, were then secured with a rotary micro-

tome, or when dealing with the more refractory tissues of the older fruits, with a Spencer sliding microtome (9).

### I. *CARYA GLABRA*

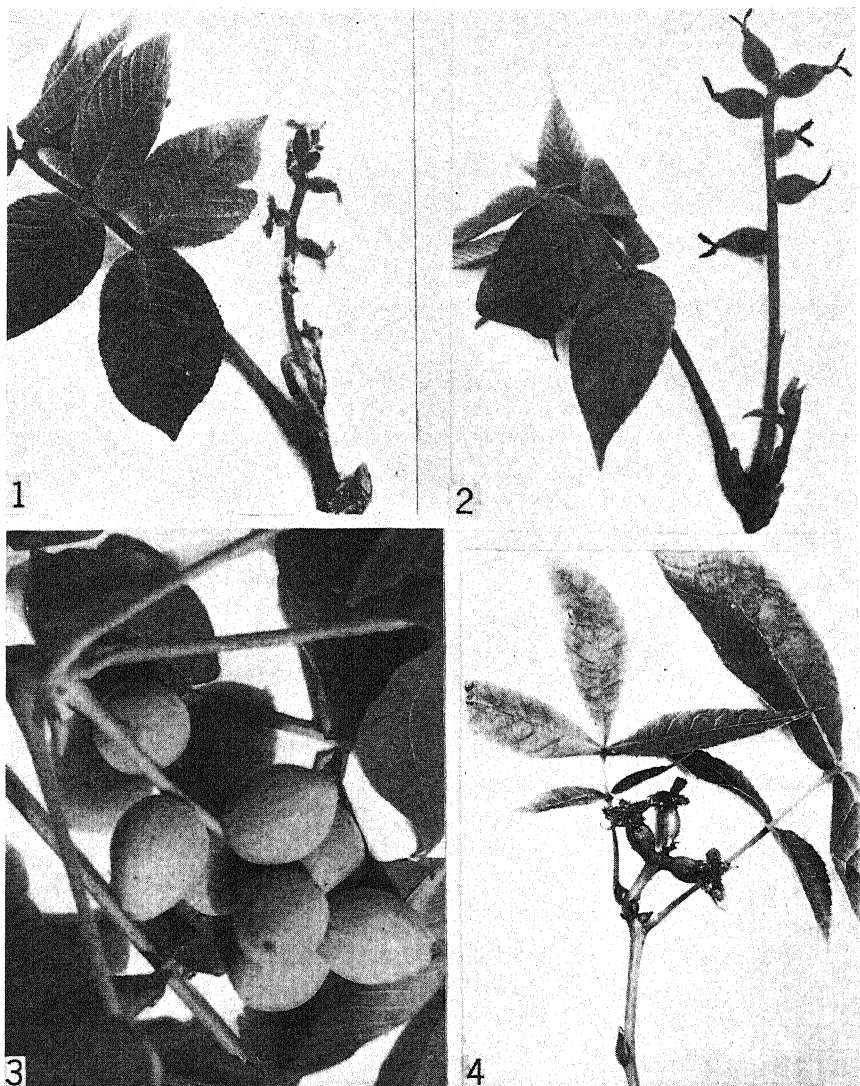
CARPELLATE FLOWERS.—The morphology of the flowers and ovules of the Juglandaceae has been treated at some length in previous papers (2, 6, 8, 15, 16, 19, 20), and will receive further and more detailed consideration in a later comparative study of this series, involving both the Juglandaceae and the Fagaceae.

In *Carya glabra* several female flowers may be differentiated on the floral axis, but only three or four of the basal or first formed continue in development, becoming visible above the leaves of the shoot early in May. The basal flowers of carpellate inflorescences collected early in May (figs. 5, 13)<sup>2</sup> exhibit characteristic floral features with calyx and stigmas well developed, some expansion of the receptacle, and ovule already inclosed by the carpels. The younger apical flowers of the clusters display partially developed calyces and short, lobelike carpels which have not as yet closed above the developing placenta. Under ordinary circumstances these apical flowers of each ovulate head do not reach maturity, but are shed shortly before or directly after pollination. Two, occasionally three, flowers of each cluster reach full development and become receptive.

Stages in ovule development observed in flower heads collected at this period (May 1–5) range from the initiation of the nucellus as a blunt, conical primordium at the tip of the columnar placenta to partially matured ovules with megasporocytes in synizesis or pachytene.

The megasporocyte is readily distinguished in the young ovules of *C. glabra*, even before initiation of the integument, as a slightly enlarged cell (the nucleus in one of the pre-synizetic phases) lying fifth or sixth in an axial strand of the nucellus. WOODROOF (22) and SHUHART (16) likewise report the presence of a definitely differentiated megaspore mother cell in the pecan, and NAWASCHIN (14) figures one in *Juglans nigra*. Within a period of six to eight days the megasporocytes of *C. glabra* pass through all the phases of synizesis and both of

<sup>2</sup> Figs. 5–12 constitute pl. I.



FIGS. 1-4.—Fig. 1, carpellate inflorescence of *J. mandschurica* 2 days after pollination. Fig. 2, same May 23, about 10 days after pollination. Fig. 3, same June 25, branch bearing nearly mature fruits. Fig. 4, same of *C. glabra*, May 25, about 10 days after pollination period.



the meiotic divisions, and have reached the megaspore stage or one of the early stages in gametophyte development at the time of pollination (figs. 25, 26). Heterotypic prophase ordinarily extends through a period of five to six days. In all material of *Carya* examined, the mother cell develops a linear tetrad of megaspores (fig. 25), the chalazal megaspore functioning as the embryo sac.

The individual ovulate flowers of *C. glabra* at pollination measure 10-12 mm. in length and 3-3.5 mm. in breadth. They are yellowish green and conspicuously glandular pubescent. The cupule (considered here as the specialized stem or enlarged receptacle) is marked in its upper section by four ridges, giving it a four-sided appearance and dividing it into four sections the margins of which are continuous with the edges of the sepals.

Longitudinal ridges or folds of the carpellary walls extend into the ovarian cavity, two of which, oriented in the plane of the carpels, grow conjointly with lateral extensions from either side of the axial placenta to form the median septum of the ovary. The problem of the septal vascular system and its relation to other bundles in the carpellate flower will be dealt with critically in a future paper. During early fruit development, all space within the ovary not occupied by the middle or primary septum and the enlarging ovule is filled with the light parenchymatous tissue of the endocarp, which takes its origin from the inner tissue of the carpel walls and from the septum. The origin of the endocarp, so-called "packing-tissue," is shown in figures 15-17. As fruit development progresses, two wedge-shaped sections of a second septum may be differentiated from the tissue of the endocarp on either side of and at right angles to the plane of the middle septum. Figure 8, a median longisection of the young fruit cut transverse to the plane of the septum, shows the expanse of parenchymatous tissue surrounding the enlarging ovule, also the initiation of the lignified tissue of the second septum at two points, one proceeding from the margins of the original septum and the other from the inner edge of the carpel walls. The two partitions, the median or original septum and the later formed second septum, divide the basal section of the ovary into four chambers which later are filled by the lobes of the expanding embryo sac and each with the half of a cotyledon.

POLLINATION.—Conflicting accounts have appeared concerning courses taken by the pollen tubes in different species of Juglandaceae. NAWASCHIN (12) found in *Juglans regia* that the pollen tubes do not pass down the stylar canal or traverse the ovarian cavity, but advance through the tissue of the style and of the ovary wall until opposite the insertion of the single ovule. They then leave the ovary wall and pierce the chalaza, branching freely in the nucellus. Male nuclei discharged into the embryo sac were seen "wandering" in its cytoplasm and fusing with one of several "free cells" that function as eggs but have not organized an egg apparatus. BILLINGS' observations (4) in *Carya olivaeformis* and NAWASCHIN'S (14) in *Juglans nigra* and *J. regia* are in essential respects in agreement with the preceding statement, except that NAWASCHIN in his later work describes and illustrates a rather well organized embryo sac with egg apparatus.

WOODROOF (22) also traces the pollen tubes of *Hicoria pecan* through the tissues of the style and ovary walls and observes that they approach the ovule opposite the plane of the middle septum, but she finds it necessary for the tubes to cross the narrow crevices which extend downward nearly to the base of the flower on either side of and parallel with the plane of the septum, and fixes the place of entrance of the tubes into the ovarian cavity as near the micropyle or at a point nearly opposite the chalaza. She finds that pollen tubes may enter the cavity of the ovary 6-12 hours after pollination, but do not enter the nucellus until the embryo sac is mature, about a week later, in the meantime growing about in the cavity and crevices.

In all the preparations of *Carya glabra* examined the nucellus is two-thirds to three-fourths inclosed by the integument at the time of pollination. Within two or three days after pollination the integument has closed firmly about the nucellus, with but a narrow crevice intervening. The pollen tubes advance through the tissue of the style, following the course of the inner set of vascular bundles to a point just above the ovarian cavity. Here they leave the vascular tissues, and, growing through the portion of the ovary wall later to be transformed into shell, pass directly to the apex of the ovarian cavity. This may require four to five days. The tip of the massive

integument is at this time in contact with the ovary wall; in fact the tissues of the two seem almost to fuse. Pollen tubes apparently invade the tissues of the integument at this point, and passing down through the integument to the base of the nucellus, approach the embryo sac from the chalaza. The interval between pollination and fertilization is slightly over two weeks, from 16 to 18 days. Pollen tubes enter the embryo sac by June 2 or 3, and always at the micropylar end of the sac.

Cases have been found in which pollination without subsequent fertilization seemed sufficient to start fruit development; in fact, fruits may reach a size of  $15 \times 7.5$  mm. and contain an ovule in which an embryo sac has never appeared.

FERTILIZATION.—Fusion of the polar nuclei precedes entrance of the pollen tube into the embryo sac (fig. 27). The primary endosperm nucleus, the dominating figure of the mature embryo sac, is usually located in the upper section of the sac (figs. 28, 29), and prior to enlargement of the sac is lodged just beneath the egg apparatus. A well defined membrane separates the egg nucleus from the synergids, with the synergid nuclei usually nearer the micropyle. Generally sections of the ovule and embryo sac made perpendicular to the plane of the middle septum show the saclike synergids overlying and partially concealing the egg (figs. 27, 28); occasionally sections cut in that plane show the orientation of the egg apparatus with both synergids appearing to one side of the egg (fig. 29). Union of the endosperm nucleus and first male nucleus is in advance of that of the egg and second male nucleus, but the interval between the two fusions is but a matter of hours, and not two to three weeks as reported by WOODROOF (22) in the pecan. The unfertilized egg invariably displays only one nucleolus; after fertilization there are two (figs. 30, 38). No internal change in the zygote is noted for a period of 17–18 days. The definitive nucleus divides immediately and endosperm accumulates rapidly in the upper section of the sac. In embryo sac material fixed June 3, 1931, two to three stages were observed: in one case a pollen tube was found taking a direct course past the egg to the endosperm nucleus with one male nucleus in contact with the primary endosperm nucleus; in other embryo sacs (slightly more advanced) remnants of a pollen tube were seen be-

tween the zygote and surviving synergid, and several endosperm nuclei in the vicinity of the zygote (fig. 30). It is not unusual in *Carya* to find at least one of the synergids surviving the passage of the pollen tube in the sac; in one case both were seen, one apparently fertilized, with the pollen tube closely applied to one side of the egg.

Through the ensuing two weeks (June 1-15) the embryo sac gradually advances on the surrounding nucellar tissue, and the endosperm is seen to form a thin but complete lining for the sac (fig. 17). Passing through the stage of free nuclei, it eventually forms cell partitions, first at the apex and base of the sac and later throughout. There may be enlargement of the embryo sac and of the entire ovule, and some development of endosperm even though a proembryo fails to appear in the sac. But expansion ceases after a period of 12-14 days, in the absence of a fertilized egg or a young proembryo, and a noticeable "drop" of fruit is found to occur at this time, about 50 per cent of the season's loss between June 12 and 15. Two similar embryos may appear in the embryo sac of *Carya glabra*, one in the position of the egg and the other in that of a synergid; only one ever passes beyond the proembryonic condition.

In regard to the synergids, NAWASCHIN and FINN (14) observed in *Juglans* that prior to fertilization not only one but both synergids become cloudy and disintegrate. So far as the fertilized egg is concerned, this remains for some time in an apparently unchanged state before its first division, often with many endosperm nuclei in the embryo sac.

EMBRYO.—The first division of the fertilized egg occurs about June 20, nearly three weeks after fertilization and five weeks after pollination. WOODROOF (22) reports the first division of the egg of the pecan eight to nine weeks after pollination, SHUHART (16) three to four weeks.

In the plasma-rich end of the elongating egg (fig. 38) two cells are separated by a wall which may be horizontal or slightly oblique. Of the two sections thus formed, the smaller apical one (*A*) contributes to the formation of the cotyledons and stem tip of the embryo, while the meristem of the root axis and the suspensor are derived from the basal section (*B*). Deviating from the procedure generally characterizing the proembryo of dicotyledons, the apex section does not

form quadrants, but is divided into three sections (fig. 39) by two vertical walls: the first one diagonal, dividing the apex cell into two wedge-shaped portions; the second also diagonal, dividing equally the larger of the two sections formed from *A*.<sup>3</sup> A third division, vertical and at right angles to the plane of the first two partitions, creates from the three sectors an apical group of six cells. From the lateral sections of this group, through subsequent sectioning, the two deeply bilobed cotyledons develop; from the central section comes the meristem out of which the vegetative point of the stem proceeds. This central section bears some resemblance to the "epiphyse" of the proembryo of *Geum* and *Myosotis* (17, 18), also to the wedge-shaped apical cells appearing occasionally in the proembryonic development of *Salix* (5).

While segmentation in the apical cell is in progress, the larger basal cell has undergone division, usually horizontal. Partitioning in the middle cell so formed is variable; nevertheless to this region can be traced the hypocotyl and the initial zone of the root tip. The basal cell formed from *B* develops the first formed portion of the root cap and the short, multicellular suspensor.

By July 11 the embryo is seen as a small spherical mass of cells inclosed by a delicately constructed cellular endosperm (figs. 42, 43). For a period of 8-10 days the embryo slowly enlarges, and by the third week in July has become a pear-shaped body (figs. 44, 45), the apical two-thirds of which is characterized by considerable meristematic activity, cell division occurring less frequently in that section of the embryo directed toward the micropyle. In the developmental stages immediately following, two meristematic areas become especially prominent: an apical one (characterized by the predominance of longitudinal divisions) from which the initials of the epicotyledonary axis take their origin, and a wedge of initials in the median third of the proembryo from which the primary meristems of the root tip are derived. For a time the periblem and plerome of the root axis develop from a common meristem, and are scarcely distinguishable as distinct regions. In the course of later embryo development, however, with the appearance of the narrow, elongated

<sup>3</sup> The first two vertical divisions are generally transverse to the plane of the carpels and the median septum of the ovary.

provascular elements, the periblem and plerome are distinctly recognizable, and long before the completion of intraseminal development become clearly differentiated right to the initials of the root apex (fig. 49). The dermatogen of the root, and a root cap-renewing initial layer are differentiated from the border of the apical meristem of the root tip.

As in *Ginkgo* (11), the primordia of the two cotyledons arise as crescentic mounds of tissue in the marginal region of the apical meristem of the proembryo (fig. 46), commencing their development almost simultaneously with organization of the meristems of root and stem tip. For a time their development precedes that of the stem meristem. By the latter part of July they are differentiated as winglike structures which develop slowly, first at right angles to and later in the plane of the middle septum, the septum eventually forming a deep partition separating the halves of the cotyledons and not the two cotyledons, as reported by WOODROOF (21) and ADRIANCE (1). Figures 47 and 50 show clearly the orientation of the primary regions of the embryo body in respect to the micropylar axis, also with reference to the middle septum, for both are at right angles to the plane of the middle septum. Most of the detailed drawings of stages in embryo development both in *Carya* and in *Juglans* have been from sections made in the plane of the middle septum, since this afforded the better view of the developing cotyledons. The bilobed character of the cotyledons becomes apparent early in August (about six weeks after the first division of the egg), when the entire embryo is little more than 1 mm. in length (fig. 50). The six primary procambial units of each cotyledon are observed to unite in pairs at the cotyledonary node or just above it, and as double strands they meet and are inserted on the desmogen of the primary root.

A broad, slightly elevated epicotyledonary axis bearing in its center three successive horizontal groups of initials (fig. 51) is recognized, surrounded by the bases of the enlarging cotyledons the latter part of July or early in August. The primordia of the first foliar organs appear without delay, and, as at the terminal apices of *Carya* seedlings (10), succeeding primordia follow one another so closely that it soon becomes difficult to distinguish the vegetative point and its initials. A plumular bud consisting of 6-8 young scale

leaves and the primordium of the first foliage leaf is organized late in August.

## II. JUGLANS MANDSHURICA

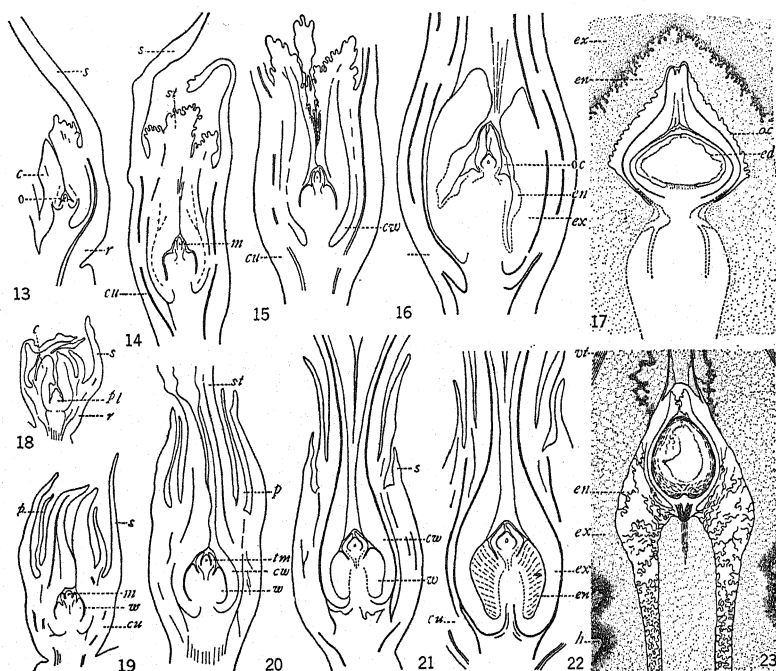
CARPELLATE FLOWERS.—Female flowers, eight to ten on a short spike terminating the shoot of the season, become visible above the leaves of the shoot early in May. Contrasting with the situation in *Carya glabra* is that found in *J. mandshurica*, in which practically all of the flowers in each ovulate head reach full development and are receptive at pollination time (fig. 1). Furthermore, a high percentage of the flowers are pollinated and continue in fruit development (figs. 2, 3).

The individual flowers are olive green, globose to ovate in form, and prior to and during the pollination period are clothed with reddish sticky hairs. Each bears two prominent brick red, plumelike stigmas which give to the carpellate inflorescence a peculiarly ornate character.

The ovulate flower is composed of two carpels inclosed by a fleshy cup-shaped receptacle or cupule, the foliar structures bordering the carpels and borne at the summit of the receptacle constituting the inner and outer members of a perianth. The order of succession of floral cycles is here interpreted as: outer members of perianth, carpels and inner members of perianth, and stamens (when present). Small abortive stamens have been observed to occur occasionally in early stages of development of the carpellate flowers of *Juglans mandshurica*. The inner set of members of the perianth do not appear in the carpellate flowers of *Carya*, nor do the stamens.

The interpretation of cupule structure suggested here is not in agreement with the generally recognized definition of the cupule as "an involucre of bracts adherent by their base." BENSON (3) has described the fertile flowers of *Juglans* as "composed of two carpels inclosed by a four-toothed calyx, and a cupule of several bracts all united in such a manner that only their upper portions are free." A detailed study of a series of stages in floral development, both in *Carya* and *Juglans* (figs. 13-17 and 18-23), has led to the conclusion that the cupule, at least of the Juglandaceae, is not to be regarded as an involucre of bracts adherent by their base, but rather as a specialized stem or receptacle, and this conclusion appears also to have

been reached by SHUHART (16) in his study of the pecan. This point will be treated at greater length in a later paper.



FIGS. 13-23.—Figs. 13-17, *Carya glabra*; figs. 18-23, *J. mandshurica*. All are median vertical sections of ovulate flowers or young fruits, collected from May 5 to June 22: Fig. 13, early stage in development of one of basal flowers showing central placenta and young ovule, differentiating carpels and enlarged outer sepal. Figs. 14, 15, floral development shortly before pollination. Fig. 16, development of flower and ovule at time of fertilization. Fig. 17, ovule about 3 weeks after fertilization. Endosperm within enlarging embryo sac is in free nuclear condition; 3-celled embryo at apex of sac. Figs. 18-20, young ovulate flowers of *J. mandshurica*. Fig. 21, flower and ovule at pollination. Fig. 22, same at fertilization. Fig. 23, about 2 weeks after fertilization showing ovule with embryo sac with parietal layer of endosperm, also 18-20-celled embryo at apex of sac. Endocarp in a partially disorganized condition. All sections cut 10-12  $\mu$  and most of them transverse to plane of septum (c, carpel; cw, carpel wall; cu, cupule; en, endocarp; ex, exocarp; ed, endosperm; h, husk; m, megasporocyte; oc, ovarian cavity; pl, placenta; r, receptacle; s, sepal; st, stigma; w, winglike evaginations of placenta).

A vertical partition, located in the plane of the stigmas and carpels, supports the single orthotropous ovule and divides the basal part of the ovary into two chambers. As in *Carya*, most of the space



within the ovary not occupied by this partition and the ovule (fig. 9) is filled with the soft white tissue of the endocarp. Unlike that of *Carya*, the endocarp in *Juglans* takes its origin as winglike outgrowths from the placenta, developing at right angles to the plane of the septum. This is well illustrated in figures 18-22, also figure 9. Shortly before pollination, and for an interval of five or six days following, the endocarp exists in a rather compact state, completely filling the ovarian cavity and crowding close about the base of the ovule (figs. 21, 22); but with growth of the ovule and later expansion of the seed coat it is forced from the vicinity of the ovule, gradually becoming broken and chaffy in character (figs. 10, 23).

One of the unique features of ovule development in *J. mandshurica*, and of somewhat frequent occurrence here, is the appearance of a double nucellus in a single ovule, a sort of bisporangiate condition, in which a single massive integument incloses both megasporangia. This condition will be found at early stages of ovule development, at the mother cell or megaspore stage, never later. The manner of origin and the ultimate fate of one or both of the nucelli in such cases have not been determined.

Ordinarily the nucellus is more than three-fourths inclosed by the integument at the time of pollination (fig. 21), and by the time of fertilization (5-6 days later) the integument completely invests the nucellus (fig. 22), growing close to the apical wall of the ovarian cavity.

POLLINATION AND FERTILIZATION.—Pollination is first evident about May 6, and four to five days are generally required for the tubes to reach the embryo sac. As in *Carya*, the pollen tubes advance through the tissues of the style following rather closely the course of the vascular bundles. The direction of growth of the tubes after reaching the vicinity of the ovary is various. They may leave the vascular tissue at this point, and, growing through the upper part of the carpel wall, pass directly to the apex of the ovarian cavity; or they may continue to follow the general course of the vascular bundles bordering the carpel wall, at intervals branching out and invading the tissue of the wall. Whatever their course in the wall of the ovary, those reaching the vicinity of the embryo sac proceed around



FIGS. 24-30.—*Carya glabra*: Fig. 24, ovule shortly before pollination, megasporocyte in pachytene;  $\times 149$ . Fig. 25, linear tetrad of megaspores;  $\times 250$ . Fig. 26, 2-nucleate embryo sac;  $\times 250$ . Fig. 27, embryo sac at union of polar nuclei. Definitely organized egg apparatus at micropylar end of sac;  $\times 149$ . Fig. 28, mature embryo sac. Primary endosperm nucleus lodged just beneath the egg, its characteristic position prior to entrance of pollen tube;  $\times 250$ . Fig. 29, mature embryo sac. Both synergids distinguishable at left of egg, one partially concealed by the other;  $\times 250$ . Fig. 30, embryo sac shortly after fertilization. Remnants of pollen tube between zygote and surviving synergid, also two of several endosperm nuclei in vicinity of zygote;  $\times 250$ .

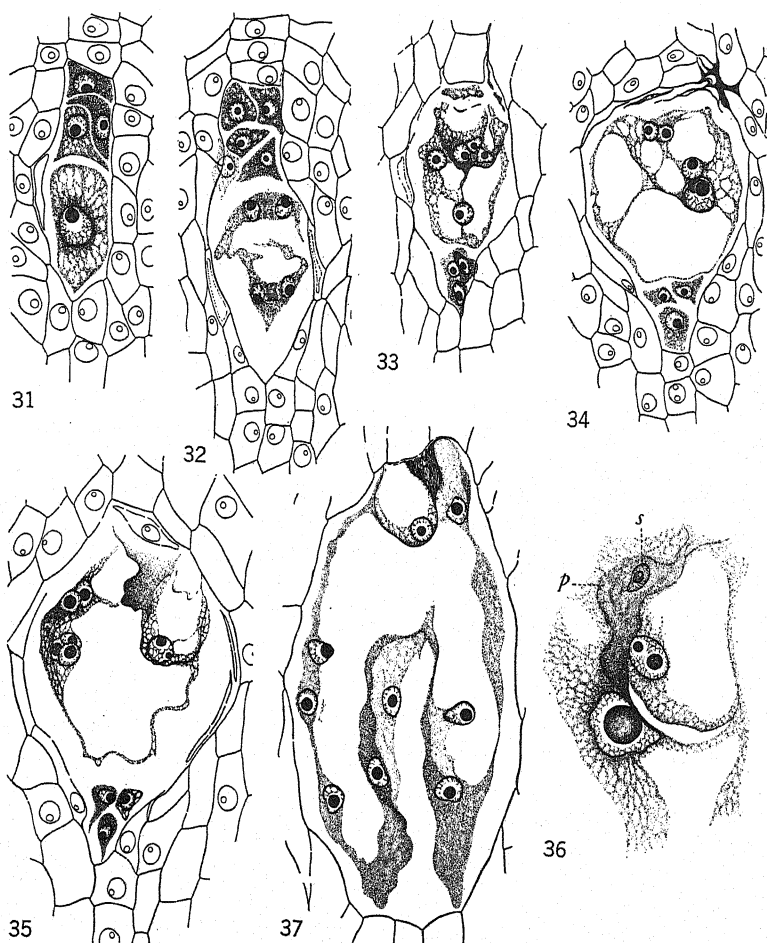
the base of the ovarian cavity and approach the ovule by way of the vascular tissues of the placenta.

The megasporocytes of *J. mandshurica* pass through the different phases of meiosis within three to four days, and embryo sacs are in one of the earlier stages of nuclear division at the time of pollination. Embryo sacs in the more advanced flowers collected May 7 of the past season were in one of the later stages of nuclear division, mostly 8-nucleate (fig. 33). In some the polar nuclei were seen in contact (fig. 34); in others, fusion of the polar nuclei had been effected. There is a noticeable shortening of the intervals here as compared with *C. glabra*, where 15-16 days were found to elapse between the megaspore stage and the mature embryo sac.

More than one pollen tube was not observed to enter the embryo sac of *C. glabra*, but in *J. mandshurica* it is not unusual to find evidence of two tubes, both entering from the micropylar end. The more common route is to one side of and in contact with the egg, usually passing between the egg and the synergids (fig. 36). One or possibly both of the synergids may be destroyed by the passage of the tube or tubes in the sac, but both are in evidence prior to their entrance, and frequently one persists after fertilization (fig. 36). This was found also to be the case in *Carya*. NAWASCHIN and FINN (14) observed several "content-conducting" tubes on their way to the egg apparatus. Sometimes the contents of one pollen tube had already emptied and located in the vicinity of the egg apparatus; at other times the contents of several pollen tubes were found around the already fertilized egg, forming a uniform mass which contained several "sperm-cell-pairs." They also reported disintegration of both synergids prior to fertilization.

In *J. mandshurica*, fertilization of the egg and the primary endosperm nucleus are not simultaneous. The male nuclei reach both at about the same time, but an interval of several hours may intervene between the two fusions. As in *Carya*, the definitive nucleus divides immediately, often before union of the egg and sperm nucleus is complete (fig. 35).

EMBRYO.—Again contrasting with the situation observed in *C. glabra*, in *J. mandshurica* only six to seven days intervene between the first division of the definitive nucleus and that of the zygote. In young



FIGS. 31-37.—*Juglans mandshurica*: Fig. 31, tetrad of megaspores, megagametophyte obviously developing from basal one;  $\times 334$ . Fig. 32, 4-nucleate embryo sac;  $\times 334$ . Fig. 33, 8-nucleate embryo sac in early stage of organization of egg apparatus. Antipodals and polar nuclei distinguishable;  $\times 334$ . Fig. 34, embryo sac prior to union of polar nuclei (lodged at right of egg, one partially concealed by other);  $\times 334$ . Fig. 35, embryo sac after entrance of pollen tube, seen applied to one side of zygote. First endosperm nuclei lodged in upper left corner of sac;  $\times 400$ . Fig. 36, embryo sac directly after fusion of egg and sperm nucleus and before first division of definitive nucleus;  $\times 500$ . Fig. 37, embryo sac several days after fertilization with abundant endosperm but zygote as yet undivided;  $\times 334$ .

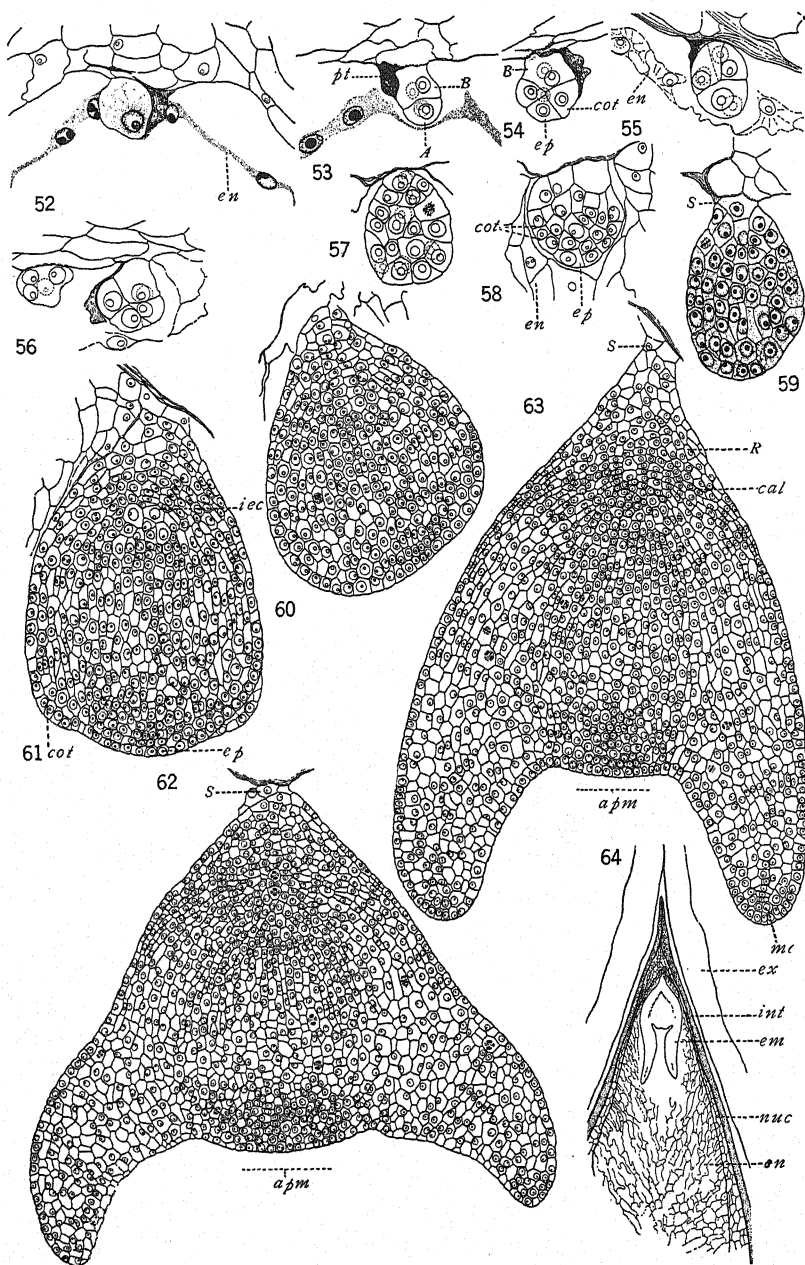
fruits collected May 18 the zygote was as yet undivided, but the endosperm was found to be in an advanced state of free nuclear division, each nucleus with two to several large nucleoli (fig. 52). A 4-8-celled embryo appears in fruits collected May 23, and by May 27 an enlarged embryo sac with a well defined parietal layer of endosperm occupies most of the nucellus (figs. 10, 23). Development and differentiation of the endosperm and embryo proceed rapidly from this point, and by June 15 (4-5 weeks after pollination) the seed coat has expanded about to full size, extending into nearly all available space within the shell, by its growth greatly enlarging the ovarian cavity. Endosperm, cellular throughout, fills the greater part of the apical section of the embryo sac, and as a layer of varying thickness lines each lobe of the sac (figs. 11, 12).

Segmentation in the proembryo of *J. mandshurica* is essentially the same as in *C. glabra*, except that greater irregularity occurs. The first division of the egg is horizontal, forming two nearly equal sections, *A* and *B*. From the apical cell (*A*) through later sectioning the cotyledons and stem tip are derived; from the basal cell the hypocotyledonary region and the suspensor. Ordinarily the apical cell is divided, as described in *Carya*, by two obliquely opposed walls (figs. 54, 56). A third division, vertical but at right angles to the plane of the first two, forms from the three sectors an apical group of six cells.<sup>4</sup> From the lateral sections of this group the two winglike cotyledons develop, and from the central section comes the meristem from which the initials of the stem proceed. Partitioning in the basal cell (*B*) is extremely variable; nevertheless in some of the clearer preparations it is possible to distinguish a primary horizontal wall in *B*. From the middle section so formed the initial zone of the root tip apparently develops; from the basal cell formed by *B* a section of the root cap and an abbreviated suspensor.<sup>5</sup> In the course of de-

<sup>4</sup> The first longitudinal division of the apex cell may be in the plane of instead of transverse to the plane of the carpels, as is the more common procedure. Furthermore, exceptions occur in which quadrant cells arise out of *A*, and obliquely placed walls in the apical quadrants separate the cotyledonary part of the embryo from the "epiphyse." The latter course also appears in *Juglans nigra*.

<sup>5</sup> Differentiation of the proembryo body into two primary regions (rather than three) corresponding to the apical and basal cells formed at the first horizontal division of the zygote is found to occur occasionally in *J. mandshurica* and is also seen in *J. nigra*. In





FIGS. 52-64.—*Juglans mandshurica*, stages in segmentation of proembryo and development of embryo. All embryos sectioned in plane of septum except fig. 62, which is transverse to that plane and shows detail of embryo seen at apex of embryo sac in fig. 12: *A*, apical section of proembryo; *B*, basal section of proembryo; *apm*, meristem of stem apex; *iec*, initials of periblem and plerome of root axis; *cal*, calyptrogen; *en*, endosperm; *cot*, cotyledonary sector; *ex*, exocarp; *in*, integument; *nuc*, nucellus; *mc*, apical meristem of cotyledons; *pt*, pollen tube; *r*, primary root; *s*, suspensor. Figs. 52-57,  $\times 188$ ; figs. 58, 59,  $\times 150$ ; figs. 60, 61,  $\times 110$ ; figs. 62, 63,  $\times 82.5$ .

velopment of the embryo the suspensor cells became so compressed as to be almost unrecognizable.

Embryological development from this point is substantially the same as outlined in *Carya*, but proceeds more rapidly. Within a period of three to four weeks, the embryo passes through all the stages in the origin and early differentiation of the primary regions of the embryo body. Orientation of the cotyledons is the same as in *Carya*, that is, transverse to the plane of the middle septum of the fruit. Their bifurcated character is early apparent, and long before the embryo in its development reaches the tip of the septum. An apical meristem is a characteristic feature of the young cotyledons (figs. 62, 63), and is retained for a time, even after their bifurcation, as an embryonic region at the tip of each cotyledonary lobe.

ENDOSPERM.—In *J. mandshurica* this is more abundant than in *Carya*. A “free nuclear” period, of four to five days’ duration, is characterized by the total absence of achromatic figures, suggesting an amitotic division of the nuclei at this time. The endosperm eventually forms a parietal plate of cells which gradually advances from the wall of the embryo sac, leaving a narrow sap-containing central region in each lobe of the sac. The cotyledons in their elongation extend between the marginal layers of the nucellus and the endosperm, in the course of their expansion gradually inclosing the inner mass of endosperm, resorbing its substance, and reducing it to a shapeless mass.

### Summary

1. The ovulate flowers both of *Carya* and *Juglans* consist of two carpels inclosed by a fleshy, cup-shaped receptacle or cupule. The foliar structures bordering the carpels and borne at the summit of the receptacle are interpreted as a perianth. Small abortive stamens occur occasionally in early stages of development of the carpellate flowers of *J. mandshurica*. In the ovulate flowers of *Carya*, neither the inner set of members of the perianth nor the stamens develop.

2. In the carpellate flowers of the Juglandaceae, an internal vertical partition (in *Juglans* and *Carya* oriented in the plane of the car-

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such cases the apex section has been observed to contribute the meristem of cotyledons, epicotyledonary axis, and even that of the root axis; the less active basal section produces the suspensor and a section of the root cap.



pels) supports the single large, sessile ovule. As fruit development progresses in *Carya*, two wedge-shaped sections of a second septum are differentiated from the tissue of the endocarp, on either side of and at right angles to the middle septum. This second septum does not appear during the development of the young fruit of *J. mandshurica*.

3. The megasporocyte is distinguishable in the young ovules, both of *C. glabra* and *J. mandshurica*, as a slightly enlarged cell lying deep in an axial strand of the nucellus, the phases of meiosis occupying six to eight days in *C. glabra*; in *J. mandshurica*, three to four days. A linear tetrad of megaspores is formed in both, the megagametophyte developing from one of the two inner megaspores.

4. In *C. glabra*, 15-16 days elapsed between the early megaspore stage and the mature embryo sac; in *J. mandshurica*, five to six days.

5. The megagametophytes of *C. glabra* and *J. mandshurica* are normal angiosperm types in their development, and alike in essential features. That of *C. glabra* is larger than the gametophyte of *J. mandshurica* and presents a more definitely organized egg apparatus. One, in some cases both, of the synergids are destroyed by the passage of the pollen tube or tubes in the sac; but both are in evidence prior to entrance of the tubes, and frequently one persists after fertilization. In *Carya* the surviving synergid may produce a small proembryo.

6. There is an interval of 17-18 days between fertilization and the first division of the zygote in *C. glabra*; in *J. mandshurica*, six to seven days. In both the definitive nucleus divides immediately, a parietal layer of endosperm being one of the distinctive features of the enlarged embryo sac prior to division of the zygote.

7. Deviating from the procedure generally characterizing the proembryo of dicotyledons, the apex cell of the young proembryo in *Carya* and *J. mandshurica* does not form quadrants, but is divided by two obliquely opposed walls into three sectors. A division, vertical and at right angles to the plane of the first two partitions, creates an apical group of six cells. In general the plane of the two cotyledons and that of the long axis of the stem tip coincides with the position of the three primary sectors of the apical section of the proembryo.

8. Segmentation in the basal section of the globular proembryo is variable, especially in *Juglans*; nevertheless to this section may ordinarily be traced the initials of the root axis, the root cap, and an abbreviated multicellular suspensor.

9. In its flower, ovule, gametophyte, and embryo features, *Juglans* may be said to present characters of greater phylogenetic interest than does *Carya*. On the other hand, the larger and more definite character of the embryo of *Carya*, both in proembryonic and embryonic phases, makes this more valuable for a histogenetic study.

The writer wishes to acknowledge her indebtedness to Dr. D. S. JOHNSON, through whose courtesy extensive collections of material were secured from the Botanical Gardens and Arboretum at Johns Hopkins University, to Dr. E. A. ANDREWS of Johns Hopkins University for a series of collections of *Juglans mandshurica*, and to Dr. C. J. CHAMBERLAIN of the University of Chicago for helpful criticism during the progress of the study.

Grateful acknowledgment is also made for two generous grants received from the National Research Council, in aid of a comparative study of the embryogeny of the Juglandaceae and Fagaceae. Valuable technical assistance has been rendered by Mr. B. L. HAMMOND.

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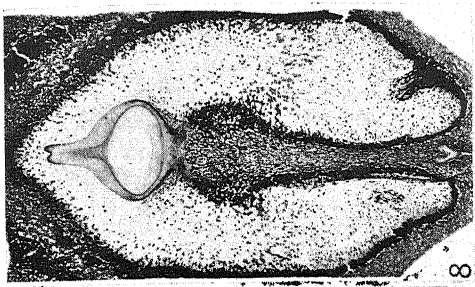
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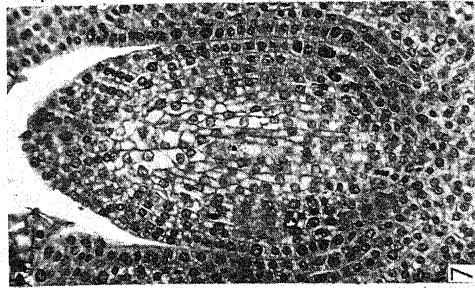
#### EXPLANATION OF PLATE I

FIGS. 5-12.—Figs. 5-8, *Carya glabra*; figs. 9-12, *J. mandshurica*.

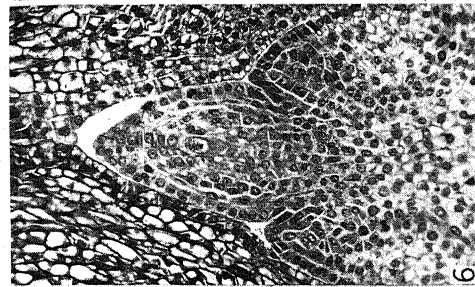
FIG. 5. Two partially matured basal flowers of ovulate inflorescence showing greatly enlarged outer sepal. Young ovule in flower located to right is at early archesporial stage.  $\times 11.8$ .



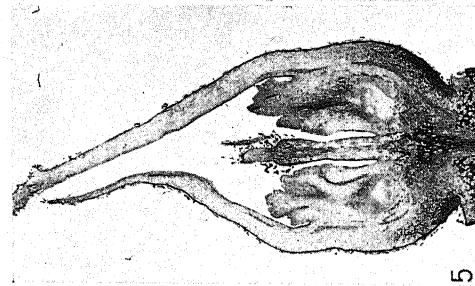
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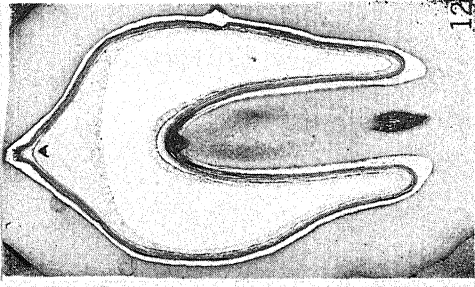
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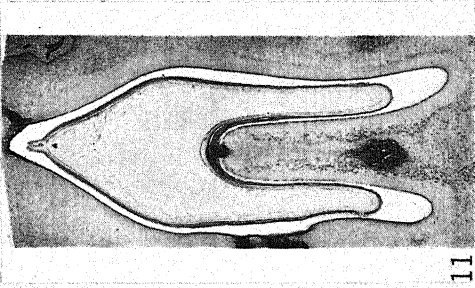
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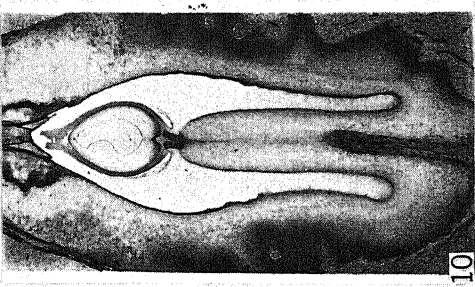
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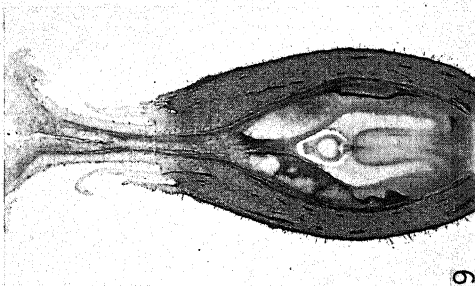
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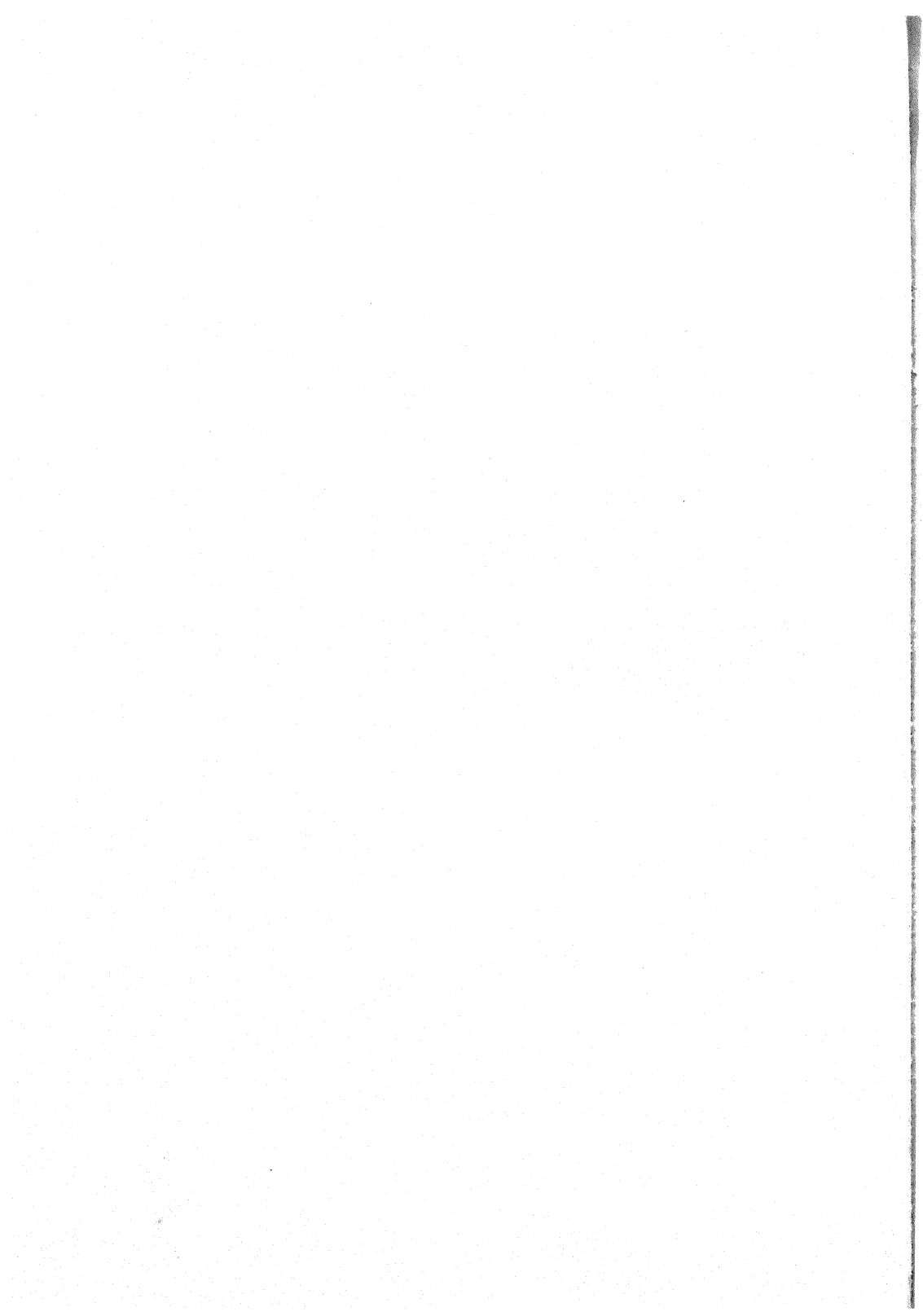


FIG. 6. Ovule at archesporial stage (megasporeocyte in synizesis).  $\times 200$ .

FIG. 7. Ovule at later stage of development (megasporeocyte in pachytene).  $\times 200$ .

FIG. 8. Median longisection of young fruit transverse to plane of median septum, showing expanse of parenchymatous tissue surrounding enlarging ovule and early differentiation of secondary septum from tissue of endocarp.  $\times 5.3$ .

FIG. 9. Median longisection of young fruit perpendicular to plane of septum, 3 days after pollination; carpellary walls and endocarp clearly defined, also vascular tissue extending between cupule and ovary wall.  $\times 5$ .

FIG. 10. Median section of young fruit perpendicular to septum, about 2 weeks after fertilization. Ovule shows embryo sac with parietal layer of endosperm, also 18-20-celled embryo. Note irregular outline of carpel wall at border of receptacle.  $\times 7$ .

FIG. 11. Median longitudinal view of nut showing embryo, endosperm, and developing testa. Embryo shows as small heart-shaped body at apex of sac.  $\times 3.3$ .

FIG. 12. Longitudinal view of nut perpendicular to septum, 5 weeks after pollination. Seed coat has expanded to about full size, extending to all available space within the shell. A well defined layer of endosperm, cellular throughout, lines the seed coat, and an embryo with diminutive cotyledons is distinguishable at apex of sac.  $\times 4$ .

# INFLUENCE OF TEMPERATURE AND NUTRITION ON THE GROWTH AND DURATION OF LIFE OF CUCUMIS MELO SEEDLINGS<sup>1</sup>

THOMAS L. EDWARDS, RAYMOND PEARL  
AND SOPHIA A. GOULD

(WITH FIVE FIGURES)

## Introduction

This paper is a report of experiments on the growth of *Cucumis melo* seedlings in darkness at five constant temperatures under aseptic conditions on agar made up either in distilled water or in Knop's solution, planned and carried out in a continuation of the seedling research program of this laboratory. Knop's mineral salt nutrient solution has been in general use for more than 60 years to supply the salt requirements of a multitude of plant species, and there was every reason to believe that in this instance, as in the others, it would induce greater growth than distilled water, as in fact it did. The specific questions which the present paper attempts to answer are these:

1. What is the relative degree of improvement in growth of seedlings of *Cucumis melo* at different temperatures with added mineral nutrients?
2. What effects do these added mineral nutrients have on the duration of life of the seedlings?

In previous publications from this laboratory, the influence of temperature on the growth and duration of life of *Celosia cristata* seedlings and on the growth of *Cucumis melo* seedlings grown under essentially the same conditions has been discussed by EDWARDS, PEARL, and GOULD (1) and by PEARL, EDWARDS, and MINER (4). The first paper (1) contains a review of the literature on the temperature relations of seedling growth.

<sup>1</sup> From the Department of Biology, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Maryland.

### Methods

The methods used in this experiment were the same as those previously described (1, 4) and found in other papers from this laboratory. They have been more fully discussed by PEARL (3) and only the essential features will be noted here. Seeds from a single melon were freed from their seed coats, weighed, and only those whose weights fell between 0.0195 and 0.0225 gm. were used. The seeds were immersed for one minute in 1:1000 HgCl<sub>2</sub> solution, rinsed once, and soaked for three hours in sterile distilled water in individual vials. They were planted in previously sterilized glass tubes 2 cm. in diameter and either 40 cm. long (for the 15° and 20° tests) or 44 cm. long (used at 25°, 30°, and 35° C.), containing 25 cc. of 1 per cent agar made up in Knop's solution in one series and in distilled water in another series. The tubes were closed with loosely fitting cotton stoppers. The seedlings were grown in darkness at the five constant temperatures just mentioned, and at 24-hour intervals from the time of the beginning of soaking the length of the hypocotyl was measured in red light.

Approximately 25 seeds were planted at each temperature. The grounds on which seeds or seedlings were removed from the experiment were: (1) failure to germinate; seven cases, four of them at 15°; (2) failure of primary root to enter the agar in the usual manner; three cases; (3) fungal contamination in two tubes; (4) abnormally slow hypocotyl growth of two seedlings; (5) so sharp a curvature of one hypocotyl that measurement became impossible. The only groups from which more than two seeds were discarded were the 15° ones.

### Results and discussion

#### INFLUENCE OF TEMPERATURE ON SIZE

Certain influences of temperature and of the two kinds of nutrition on the development of *Cucumis* seedlings are illustrated in figure 1, which is arranged to combine the functions of illustration and graph and which shows the appearance of representative seedlings of each of the ten groups of seedlings at the time they reached their final size. At the time growth ceased in each group, a seedling whose hypocotyl had the same length as the mean of the group re-



ceiving the same treatment was selected to show typical root development, and was photographed at a standard distance from the

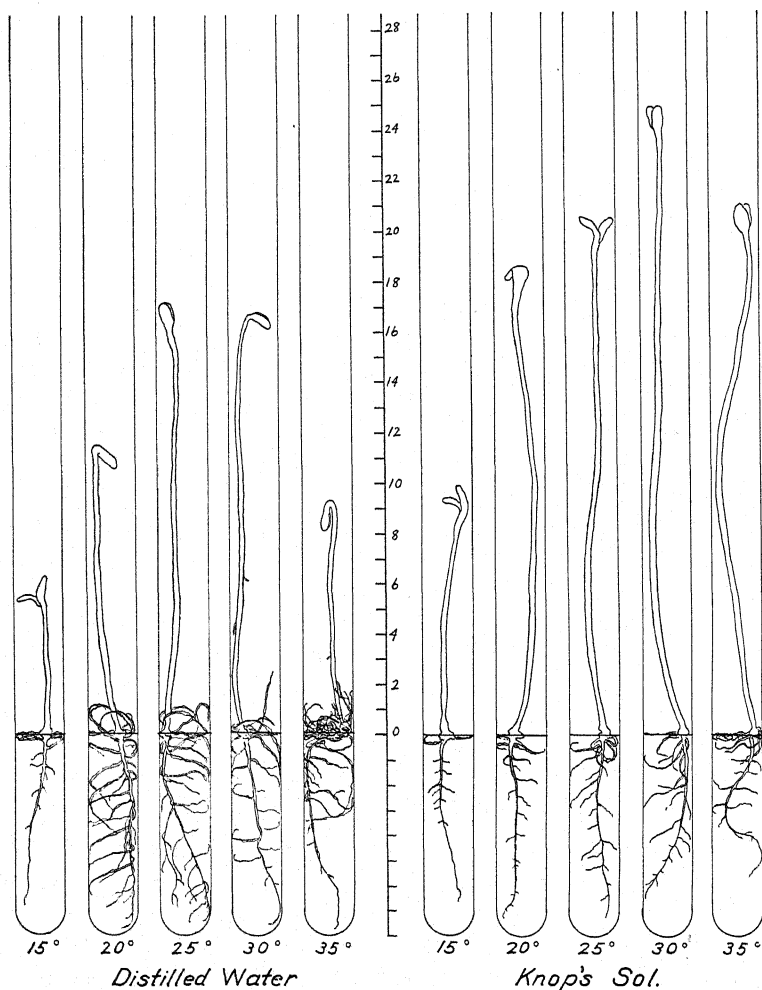


FIG. 1.—Representative *Cucumis melo* seedlings grown at five temperatures and with two kinds of nutrition at the time final size was attained. Scale reads in centimeters.

camera. Drawings were prepared from these photographs to the same scale.

In both series the greatest amount of hypocotyl growth occurred

at 30°, just as in another series of tests on seedlings of this species already reported (4), and the yields at the other temperatures fall along the usual type of optimal temperature curve. There are several conspicuous differences between growth on distilled water and growth on Knop's solution which figure 1 serves to illustrate. Considering each temperature separately, the hypocotyls grown on agar made up in the mineral salt solution are definitely taller than are those on plain agar. This difference is most pronounced at the temperature extremes, 15° and 35°, where the difference is almost 2:1, and it is least pronounced in the optimal temperature region at 25° and 30°. This is the same sort of relation as reported by TOMKINS (6) for the temperature relations of growth of several species of fungi in the presence or absence of nutrients, and which he expressed in the following words (p. 399): "At this stage in the enquiry a broad principle seems to emerge, namely, that when any one of the conditions is modified so as to favour growth, that is make it more rapid, the limitation of the growth rate by variation of any of the other conditions is rendered more difficult." "It would follow from this principle that any condition tending to retard growth . . . would have least effect at the optimum temperature."

During the course of the measurements it became apparent that some groups of seedlings were more variable in height than others. The standard deviations and coefficients of variation of the final heights, together with their probable errors, are presented in a later section of this paper, and indicate which treatments resulted in relatively uniform seedlings and which were more variable in final height. To anticipate briefly these results here, it appears that in general the higher temperatures tend to have larger standard deviations than 15°; that is, there is a greater scatter of the individual seedling heights from the means. Such temperatures also produced taller seedlings, however, and the greater absolute heights create greater numerical differences among the seedlings. The coefficient of variation minimizes this discrepancy by reducing the measure of variation to a percentage basis, and comparison of the values for this constant shows that the greatest degree of uniformity was found in the optimal temperature range noted in the discussion of figure 1, 25° and 30° in the distilled water series, and 25°, 30°, and 35° in the

mineral nutrient solution series. These coefficients are somewhat low for biological material. The extremes of the temperature range in the distilled water series and the lower temperatures of the Knop's solution series produced the most variable seedlings. The coefficients of variation are high in the early stages of growth and fall rapidly at first.

The more vigorous hypocotyl growth on Knop's solution is accompanied by meager root growth, as compared with the distilled water series. The total amount of growth made by the roots does not show the same relation to temperature as hypocotyl development does; in fact, there seems to be an inverse relation between the two. In the distilled water series the heaviest root growth occurred at 20°, and this is confirmed by the dry weights. On account of the scanty root development on Knop's solution, the small differences in final size are not evident in the drawings. To judge from their dry weights, however, the greatest amount of root growth was found at 15°.

Often the junction of the root and the hypocotyl was raised above the level of the agar, and many secondary roots, especially in the distilled water series, did not enter the agar at all. Short adventitious roots appeared high up on numerous hypocotyls grown at 30° and 35° on distilled water agar, but they were not found under any other conditions of the experiments.

There is another kind of relationship (a minor one not clearly shown in figure 1 and one for which quantitative measurements are lacking) which fits into this scheme of internal regulations, namely, the degree of development of the petioles of the cotyledons. At high temperatures they are short; at low temperatures they are longer and appear to be extensions of the hypocotyl.

The means of the daily measurements of hypocotyl length are set forth in table I (A and B) and they appear in figure 2 as a pair of three-dimensional diagrams, one for the distilled water series and one for the Knop's solution series. These are constructed as though the growth curves for each of the five temperatures have been drawn on some material like cardboard, cut out, and set upright in proper order. At right angles runs another series of planes which may be regarded as indicating the relative heights of the hypocotyls 2, 4, 6

... days after planting. The planes on which the growth curves are drawn are shown extended to the time of the beginning of death of the hypocotyls. To facilitate comparisons, numerals have been

TABLE IA  
MEAN HEIGHTS IN CENTIMETERS OF CUCUMIS MELO HYPOCOTYLS  
FOR DIFFERENT INTERVALS AFTER PLANTING; DISTILLED WATER

DAYS	15°	20°	25°	30°	35°
2			0.22	1.14	0.81
3			1.15	3.44	1.80
4			2.98	5.72	3.15
5		0.33	5.12	8.23	4.25
6		0.91	7.13	10.95	5.47
7		1.65	9.59	12.92	6.64
8		2.88	11.43	14.36	7.22
9		4.39	12.93	14.97	7.79
10		6.04	14.15	15.57	8.19
11		7.21	14.89	15.94	8.46
12	0.17	8.32	15.26	16.20	8.72
13	0.30	8.94	15.47	16.39	8.85
14	0.40	9.46	15.58	16.44	8.88 ± .13
15	0.52	9.93	15.63 ± .09	16.45 ± .10	
16	0.64	10.36			
17	0.75	10.45			
18	0.95	10.67			
19	1.17	10.81			
20	1.39	10.86			
21	1.71	10.88 ± .11			
22	2.02				
23	2.33				
24	2.76				
25	3.21				
26	3.38				
27	3.68				
28	3.89				
29	4.09				
30	4.24				
32	4.36				
34	4.49				
36	4.61				
38	4.67				
40	4.70 ± .07				

placed on the graphs to indicate the actual final heights and durations of life of each group.

From these data it appears that in both series throughout the entire growing period the 30° hypocotyls were taller than those grown at any other temperature. In every instance the plotted points fall along S-shaped curves which rise a little more abruptly from the

base line in the distilled water series than in the Knop's solution series. This difference in form and the differences due to temperature will be discussed later in this paper. There is a tendency for growth to begin a little earlier on Knop's solution and to be terminated sooner than on plain agar. Considering each temperature

TABLE IB

MEAN HEIGHTS IN CENTIMETERS OF CUCUMIS MELO HYPOCOTYLS FOR  
DIFFERENT INTERVALS AFTER PLANTING; KNOP'S SOLUTION

DAYS	15°	20°	25°	30°	35°
2.....			0.34	1.59	0.78
3.....		0.24	1.73	5.30	2.29
4.....		0.81	4.67	9.84	4.74
5.....		1.89	7.97	15.71	8.06
6.....		3.79	11.91	19.85	11.96
7.....		6.39	14.33	21.76	15.09
8.....		9.46	16.02	22.62	17.14
9.....		12.29	17.15	22.94	18.52
10.....		14.49	17.92	23.03	19.04
11.....		15.73	18.34	23.07	19.46
12.....	0.46	16.41	18.58		19.66
13.....	0.63	16.74	18.68±.17	23.10±.20	
14.....	0.77	16.86			19.72±.16
15.....	0.91	16.97			
16.....	1.06	17.03			
17.....	1.37	17.07			
18.....	1.68	17.08±.17			
19.....	2.14				
20.....	2.80				
21.....	3.70				
22.....	4.69				
23.....	5.51				
24.....	6.28				
25.....	6.80				
26.....	7.15				
27.....	7.47				
28.....	7.70				
29.....	7.80				
30.....	7.88				
32.....	7.98				
34.....	8.04±.11				

separately, the distilled water series was longer lived than the better nourished series; and in each series duration of life was, with certain exceptions to be noted later, inversely proportional to temperature.

Unfortunately, during the tests on Knop's solution at 25°, the temperature control went out of order on the sixth day and the temperature fell to 23° for the rest of the growth period; this seems to

have reduced the final height somewhat in comparison with the performance at higher and lower temperatures.

Any attempt to compare the forms of the growth curves constructed directly from the data of table I is made difficult by the

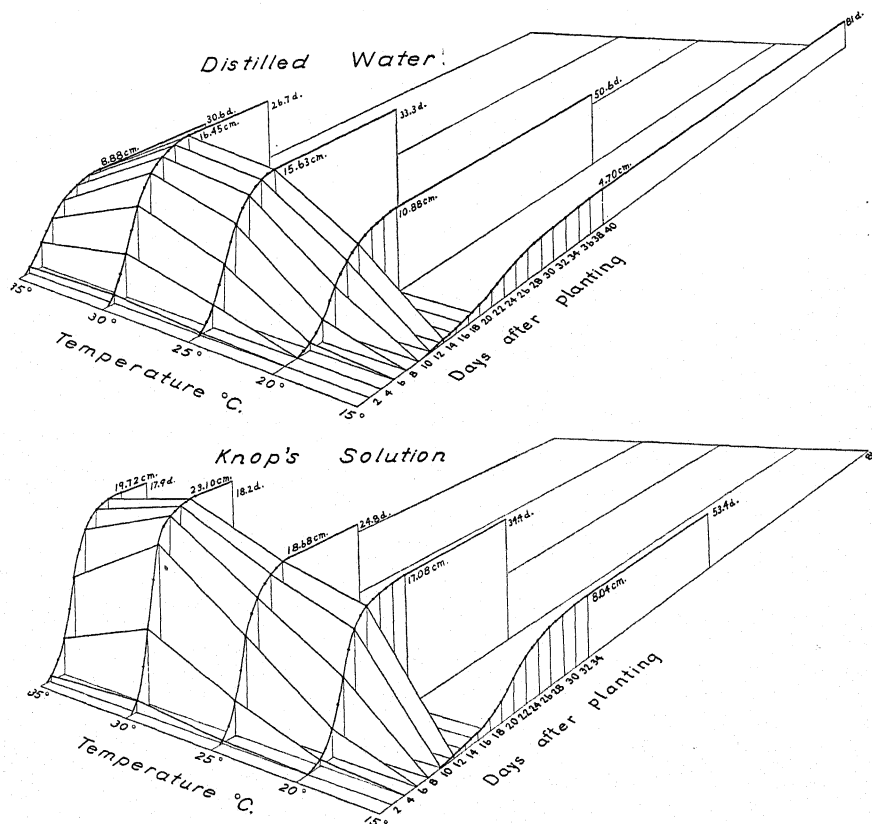


FIG. 2.—Three-dimensional diagrams showing course of growth and duration of life of *Cucumis* seedlings grown at five temperatures.

circumstance that the curves cover different time spans and in addition differ greatly in final size, as figures 1 and 2 illustrate. A simple arithmetical procedure introduced by PEARL, EDWARDS, and MINER (4) has been used to bring the ten curves into uniform scales of time and relative growth. The time from planting to the cessation of growth was taken as 100 per cent in each case, and the relative

heights for 10, 20, 30 . . . per cent of the growing period were calculated by dividing the heights for these fractional growth periods by the final height, and multiplying by 100. Thus each curve starts at 0 on each scale and ends at 100. An example will illustrate this procedure. At 20° on Knop's solution, growth was considered to have ceased on the seventeenth day after planting. By interpolation, assuming that growth proceeded at a uniform rate between each pair of observations, the heights for 1.7, 3.4, 5.1 . . . days were calculated (these periods constitute 10, 20, 30 . . . per cent of the

TABLE II  
APPROXIMATE RELATIVE YIELDS (MEAN HYPOCOTYL LENGTHS)  
AT STATED RELATIVE TIMES

GROWTH PERIOD (PER CENT)	PERCENTAGES OF OWN TOTAL YIELD									
	DISTILLED WATER SERIES					KNOP'S SOLUTION SERIES				
	15°	20°	25°	30°	35°	15°	20°	25°	30°	35°
10 . . . . .	.....	.....	1.0	5.2	6.4	.....	.....	1.2	3.8	2.8
20 . . . . .	.....	.....	7.4	20.9	18.1	.....	2.7	6.3	10.1	10.1
30 . . . . .	3.6	8.4	25.9	42.3	37.9	.....	12.2	23.4	28.9	27.4
40 . . . . .	13.6	26.5	45.6	66.6	56.1	8.8	34.4	46.9	52.8	52.7
50 . . . . .	29.6	55.6	67.2	82.8	74.8	17.0	63.7	70.2	77.1	76.5
60 . . . . .	58.7	76.6	82.7	91.0	83.9	39.3	86.3	83.9	91.0	89.7
70 . . . . .	82.8	87.1	92.9	95.6	91.3	76.2	95.7	92.2	96.9	96.2
80 . . . . .	92.8	95.4	97.6	98.4	95.8	93.5	98.5	97.4	99.2	98.9
90 . . . . .	98.1	98.3	99.3	99.7	99.1	98.4	99.6	99.1	99.8	99.8
100 . . . . .	100	100	100	100	100	100	100	100	100	100

growing period), these interpolated heights were divided by 17.07 (the height on the seventeenth day), and the quotients were multiplied by 100. These data are shown in table II and in graphical form in figure 3 as two families of sigmoid curves.

The distilled water curves have different degrees of symmetry; the low temperature curves do most of their growing relatively late in the growth period, the high temperature curves do most of their growing early. At 15°, for instance, less than one-third the final height was attained during the first half of the growth period, but at 30° more than four-fifths of the total growth had been accomplished in the same relative time. At 20°, 56 per cent of the total growth had

been completed at the midpoint of the growth period; at  $25^{\circ}$ , 67 per cent. At the only supra-optimal temperature tested,  $35^{\circ}$ , the value for the first tenth of the period is a little greater than the value for  $30^{\circ}$ , but thereafter growth was slower than at  $30^{\circ}$ .

The course of growth of seedlings supplied with mineral nutrients seems to have been much less affected by differences in temperature, to judge from the curves for the four higher temperatures in Knop's solution series. The curves for  $30^{\circ}$  and  $35^{\circ}$  take almost the

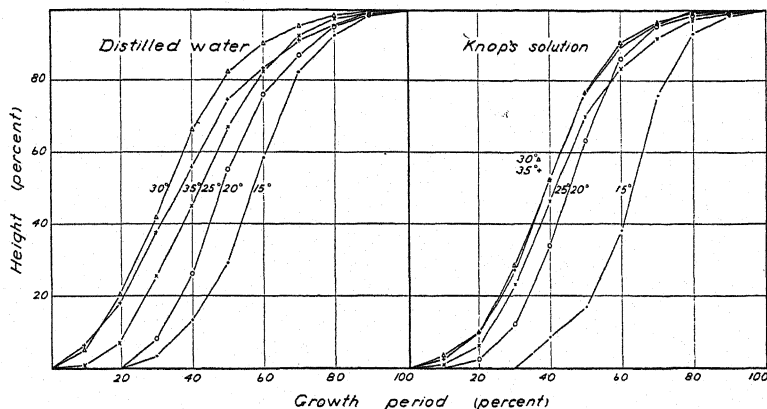


FIG. 3.—Growth curves showing percentages of final heights attained at expiration of various fractions of growth period.

same course, and those for  $20^{\circ}$  and  $25^{\circ}$  are not widely different. This is in agreement with the principle noted in the discussion of figure 1, that with improvement of the conditions affecting growth the influence of other modifying factors is lessened. Elongation began late in  $15^{\circ}$  and the growth curve is correspondingly delayed. The five curves of this series rise less steeply during the early periods than do the distilled water curves, but they compensate for this by relatively greater growth rates in the latter half of the growing period, a relationship which can be seen in figure 2 also.

#### DRY WEIGHTS

Several details of the experimental procedure need to be kept in mind in considering the dry weights presented in figure 4: (1) Seedlings were removed one by one as the hypocotyl of each showed the



first signs of death,—usually constriction of a zone near the cotyledons or the filling of the intercellular spaces of some part of the hypocotyl with water, giving it a translucent appearance. (2) Each seedling was cut up into cotyledons (including their petioles), hypocotyls, and roots, and the separate organs were dried at 98° and weighed. (3) The seedlings were grown under aseptic conditions throughout the entire experimental period and as a result they died from starvation rather than from bacterial or fungal attack. (4)

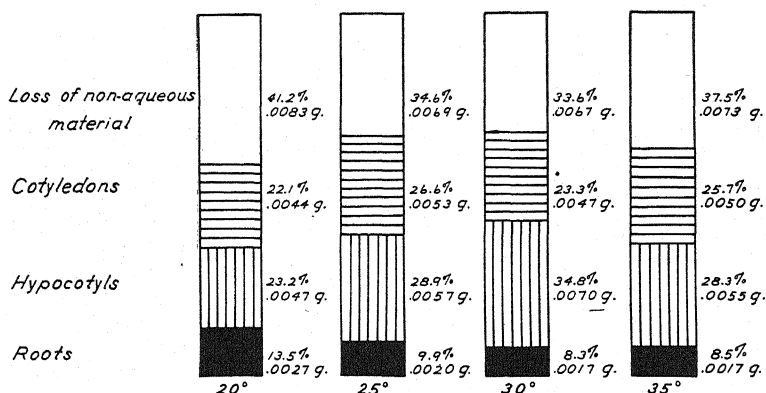


FIG. 4.—Bar diagrams showing relative amounts of dry matter found in cotyledons, hypocotyl, and roots at beginning of death and the proportion used in respiration at four temperatures in the distilled water series.

The seedlings grown on distilled water and agar did not receive any external sources of energy and materials except water and oxygen. Agar is not utilized, as unpublished tests show. As a result the amount of material available for growth and the maintenance of life was restricted to the store of food materials present in the dry seed. In this series the weights may be used directly as an index of the amount of non-aqueous materials remaining at the time of the beginning of death, and they are so treated in the bar diagrams of figure 4. (5) The seedlings grown on Knop's solution were able to absorb salts, and the dry weights represent both non-aqueous organic materials and the unknown amount of salts and so they are of no use in this connection. Dry weights are not available for the distilled water seedlings grown at 15°, unfortunately.

Each seed was weighed individually before planting and the total seed weights used for producing each of the ten experimental groups of seedlings are known. Of the seed weight, 95.49 per cent consisted of non-aqueous materials; and from the dry weights of cotyledons, hypocotyls, and roots the proportions of the original material laid down in these three organs were calculated on a percentage basis. These values are expressed graphically and numerically in figure 4, accompanied by the observed values for dry weights. The sum of the dry weights of the three organs never equaled the original dry seed weight of course; during the life of the plant respiratory processes continually produced water and carbon dioxide, which diffused away, part of their weight probably being compensated for by the weight of the oxygen absorbed. To obtain a measure of the metabolic activity of the seedlings, the total dry weights of the dead plants were subtracted from the calculated dry weights of the seeds used to produce the plants; this quantity is also expressed in figure 4.

The upper sections of the bars, representing the loss in material due to respiratory processes, show a remarkable relationship; the lowest values occur in the optimal temperature range at 25° and 30°. This is remarkable because numerous experimenters working on a variety of kinds of organisms have found that as successively higher temperatures are examined, higher respiratory rates are found; and on that basis one might have predicted that the greatest loss of non-aqueous materials would have been found at the highest rather than at the lowest temperature studied.

WASNIEWSKI'S (7) results on the metabolism of wheat seedlings are of interest in this connection. He grew seedlings to the stage when the third leaf began to unfold at 10°, 20°, and 34° C. on distilled water, Knop's solution, and a N-free salt solution, and made detailed chemical analyses of seeds and seedlings. On the average, about 72 per cent of the starch was used in respiration at 10° and 20° and 82 per cent at 34°, indicating a less economical use of materials above the optimum. Reserve materials were used less economically on distilled water than on Knop's solution by the wheat seedlings. WASNIEWSKI'S experiments were terminated at a much earlier developmental stage than were the present ones.

The highest hypocotyl weights came at 30° and the values for the

other temperatures grade off much as do the heights of the hypocotyl. The root weights were greatest at 20° and decrease with higher temperatures. The bars show that the weights of hypocotyl and roots combined, representing the total production of new tissue, was greatest at 30°. No similar trends are shown by the cotyledons. It appears that the beginning of death found the cotyledons of each group depleted of food materials to about the same extent, both relatively and absolutely.

When the proportions of dry material deposited in the three organs are expressed as percentages of the total dry weight of the seedling (as in table III) instead of on the basis of the original dry weight

TABLE III  
PERCENTAGE DISTRIBUTION OF DRY MATERIAL IN PLANTS AT TIME OF  
BEGINNING OF DEATH; DISTILLED WATER SERIES

PART	20°	25°	30°	35°
Cotyledons.....	37.6	40.7	35.1	41.1
Hypocotyl.....	39.5	44.1	52.4	45.2
Roots.....	22.9	15.2	12.5	13.6
Totals.....	100.0	100.0	100.0	99.9

of the seed (as in figure 4), very much the same relationships are found.

Except for the entrance of oxygen and the escape of carbon dioxide and water vapor through the cotton stoppers of the tubes, the seedlings were operating in what may be considered to be a closed system; and under the conditions of this experiment the amount of material at the disposal of the embryos was definitely limited to the amount stored in them and in their cotyledons. This material was used almost exclusively, one may suppose, in three ways: for the production of a root system, for the production of a hypocotyl, and expended in respiration. If one selects a set of environmental conditions in which one of these processes is accelerated, it is clear that this can occur only at the expense of one or both of the other processes, provided that the same absolute amount of material in total is taken in each and every case from the available cotyledonary store, under all

of the tested experimental conditions, as appears to have been the case in the present experiments. This principle was expressed in 1822 by GEOFFROY (2, p. xxxiii) who wrote, "an organ, normal or pathologic, never acquires an unusual prosperity without a related organ, or one in the same system, suffering for it." The matter can be stated in the opposite way, also. If one selects environmental conditions such that one of these processes is inhibited, then the amount of material available for the remaining processes is conserved and they would appear to have been stimulated. In this sort of system one cannot stimulate or inhibit one function of the organism separately without effect on the others; by choice of suitable temperature and kind of nutrition one can shift the balance of the three major food-consuming processes in this way or that. If temperature is the means used for creating environmental differences in a series of experiments, by this means shifting the internal balance of physiological processes, it is possible to speak of one temperature or another as being optimal; it all depends upon the process being studied.

Another means of altering the balance of physiological processes is illustrated by these data. If nutrient salts are supplied to the seedlings, hypocotyl growth is accelerated, relative to the performance on distilled water, but it is accomplished at the expense of root growth.

#### DURATION OF LIFE AND RATE OF LIVING

Three types of experiments have shown an inverse relation between growth rate and duration of life in seedlings grown under the same conditions as these.

1. Taking the normal variation in growth rates and in durations of life existing in series of 46 seedlings exposed to the same environmental conditions, PEARL (3) worked out the coefficient of correlation, finding it to be, in two different experiments,  $-0.463 \pm .078$  and  $-0.643 \pm .057$  respectively, indicating that high growth rates are associated to a statistically significant degree with short durations of life. From the results of experiments on *Drosophila* and from consideration of the literature there reviewed, PEARL was led to the conclusion that "in general, the duration of life varies inversely as the rate of energy expenditure during its continuance. In short, the length of life depends inversely on the rate of living."

2. PEARL, EDWARDS, WINSOR, and WINSOR (5) found that when differences in growth were created in *Cucumis* seedlings by differences in aeration, the same relation between growth rate and life duration appeared.

3. In experiments similar to those reported here but conducted with *Celosia cristata* seedlings, EDWARDS, PEARL, and GOULD (1)

TABLE IV  
HYPOCOTYL LENGTH, DURATION OF LIFE, AND GROWTH RATE  
OF CUCUMIS SEEDLINGS

	TEMPERATURE				
	15°	20°	25°	30°	35°
<i>Distilled water</i>					
Mean hypocotyl height, cm.....	4.70 ± .07	10.88 ± .11	15.63 ± .09	16.45 ± .10	8.88 ± .13
Standard deviation....	0.40 ± .05	0.77 ± .07	0.64 ± .06	0.75 ± .07	0.93 ± .09
Coefficient of variation	8.51 ± .99	7.05 ± .69	4.06 ± .40	4.56 ± .43	10.49 ± 1.08
Duration of growth, days	40	20	15	15	14
Duration of intermediate period.....	41	30.6	18.3	11.7	16.6
Duration of life, days...	81	50.6	33.3	26.7	30.6
Mean growth rate, cm./day.....	0.118	0.544	1.042	1.097	0.634
Number of seedlings....	17	24	24	26	22
<i>Knop's solution</i>					
Mean hypocotyl height, cm.....	8.04 ± .11	17.08 ± .17	18.68 ± .17	23.10 ± .20	19.72 ± .16
Standard deviation....	0.73 ± .07	1.13 ± .12	1.12 ± .12	1.36 ± .14	1.18 ± .11
Coefficient of variation	9.04 ± .95	6.61 ± .69	5.99 ± .64	5.90 ± .62	5.98 ± .57
Duration of growth, days	34	17	13	11	14
Duration of intermediate period.....	19.4	17.4	11.8	7.2	3.9
Duration of life, days...	53.4	34.4	24.8	18.2	17.9
Mean growth rate, cm./day.....	0.237	1.005	1.437	2.100	1.409
Number of seedlings....	21	21	20	21	25

found considerable differences in growth rate among six temperatures tested, but in general high growth rates were accompanied by short life durations, and slow growth at the low temperatures was followed by long life.

Table IV and figure 5 show the interrelationship of these two functions in the set of data under discussion here. The growth rates have been obtained by dividing the final height in cm. by the duration of

the growth period in days. In the mineral nutrient series the values increase to a maximum at  $30^{\circ}$ , as was reported previously for this species by PEARL, EDWARDS, and MINER (4), who have discussed this method of calculating the mean growth rate in comparison with other measures. In the distilled water series the rates at  $25^{\circ}$  and  $30^{\circ}$  are practically the same and they are higher than at the other temperatures. For comparison with this there are two measures of life duration: (1) the period from planting to the beginning of death, which is greatest at the lowest temperature tested and decreases in

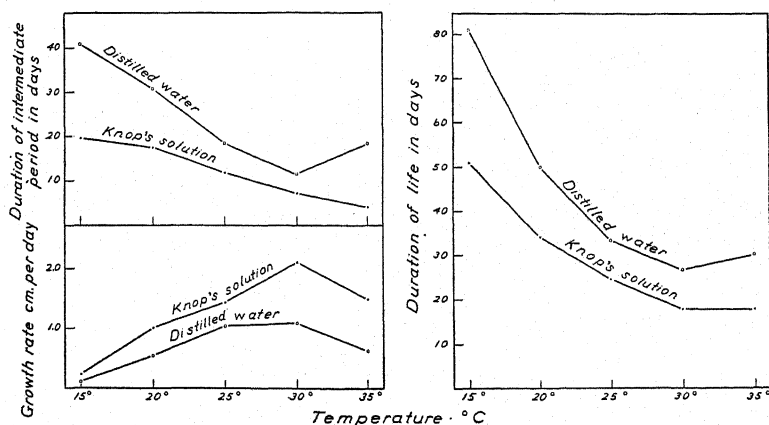


FIG. 5.—Total duration of life, duration of intermediate period, and mean growth rates of *Cucumis* seedlings.

both series to low values for the highest temperatures. (It appears that seedlings lived a little longer at  $35^{\circ}$  than at  $30^{\circ}$  on distilled water, but no great importance can be attached to this on the basis of the data available at present.) (2) Since the growth period makes up a substantial part of the total duration of life, the growth rate and total duration of life are not independent variables, arithmetically. Consequently, as in the other papers from this laboratory already referred to, the length of the growth period was subtracted from the duration of life to give the length of time during which the seedlings maintain themselves in an apparently healthy condition but without further change in size (referred to here as the intermediate period). These data fully confirm the conclusions drawn from the earlier ex-

periments in showing an inverse relation between growth rate and life duration.

The two environmental conditions used here as independent variables, namely temperature and nutrition, influence both growth rate and life duration. In general, high temperatures accelerate growth in both series and they shorten life. The addition of salts to the agar also accelerates hypocotyl growth at all temperatures to about twice the rates on distilled water, but the duration of life is reduced at all temperatures also.

This study of the behavior of *Cucumis* seedlings has included only the most easily measured characters, but the observations show that the logically separable functions of the plant, such as hypocotyl growth, or life duration, cannot be altered by suitable choice of environmental conditions without modifying the other activities of the organism also; and in the system set up here one function can be accelerated only at the expense of a complementary function.

### Summary

1. *Cucumis melo* seedlings were grown under aseptic conditions on distilled water or on Knop's solution at five constant temperatures between 15° and 35° C., and measurements were made on (1) growth of the hypocotyl, which was found to be greatest and most rapid at 30°; (2) duration of life, which was found to be inversely proportional to temperature; and (3) dry weights of cotyledons, hypocotyl, and roots at the beginning of death.

2. The differences between hypocotyl growth on nutrient and on plain agar were greatest at the temperature extremes and least in the optimal temperature range.

3. The greatest hypocotyl growth occurred on Knop's solution but root growth was poor, compared with distilled water; the optimal temperature for hypocotyl growth was 30°, for roots 15° or 20°; rapid growth was followed by short life duration.

4. In the ten sets of environmental conditions, roots and hypocotyls had different degrees of development and the amount of non-aqueous materials lost in respiration during the life of the plant dif-

ferred also. It appears that these processes are so interrelated that any excessive development of one function is counterbalanced by a reduced activity of another function.

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SOME NEW OR OTHERWISE NOTEWORTHY MEMBERS  
OF THE FAMILIES LABIATAE AND COMPOSITAE

EARL EDWARD SHERFF

**Haplostachys linearifolia** (Dr. del Cast.) comb. nov.— *Phyllostegia linearifolia* Dr. del Cast. Illustr. Fl. Ins. Mar. Pac. 47, tab. XX. 1886.

HAPLOSTACHYS LINEARIFOLIA var. **rosmarinifolia** (Hillebr.) comb. nov.— *H. rosmarinifolia* Hillebr. Fl. Haw. Isls. 347. 1888.<sup>1</sup>

Differs from the species proper in having narrower, more elongate-acute calyx lobes, these averaging 2–3.3 mm. rather than 1–1.5 mm. long.

**Phyllostegia mannii** nom. nov.—*Stenogyne parviflora* H. Mann, Proc. Amer. Acad. 7:193 (Enum. Haw. Pl. no. 357). 1867.—Very properly regarded by HILLEBRAND (Fl. Haw. Isls. 353. 1888) as a *Phyllostegia*, but confused by him with *P. racemosa* Benth., a quite different species from the Islands of Hawaii and Molokai. *P. mannii* is known only from the Island of Maui. The lower lip of the corolla exceeds the upper, as in other true species of *Phyllostegia*.

PHYLLOSTEGIA GLABRA var. **macraei** (Benth.) comb. nov.— *P. macraei* Benth., DC. Prodr. 12:554. 1848; *P. glabra* var.  $\beta$ . Hillebr. Fl. Haw. Isls. 351. 1888.

PHYLLOSTEGIA GLABRA **lanaiensis** var. nov.—Folia angustiora late oblongo-lanceolata, sub 4 cm. lata, subobscure serrata, sicca subnigrescentia. Calyces ad anthesin obconici (lobis acriter subulatis irregulariter usque ad 7 mm. longis inclusis) circ. 1–1.1 cm. longi; corollis circ. 1.5–1.7 cm. longis, tubo paulo exserto.

**Specimens examined:** *Mr. Ballieu*, Hawaiian Isls. (Herb. Par.); *George C. Munro*, Isl. Lanai, June 20, 1914 (Herb. U.S. Nat.); *H. Mann* and *W. T. Brigham* 354, Isl. Lanai (type, Herb. Field Mus.).

PHYLLOSTEGIA MACROPHYLLA **remyi** var. nov.—Folia glabriuscula vel moderate adpresso-hispida. Calyces floriferi tantum circ. 2.5 mm.

<sup>1</sup> *Phyllostegia rosmarinifolia* H. Mann, Mem. Bost. Soc. Nat. Hist. 1:536. 1869 (nom. nudum) evidently belongs either to this variety or to the species proper.

longi, lobis sub 0.5 mm. longis, pedicellis 6-8 mm. longis, corollis minoribus vix 1 cm. longis.

**Specimens examined:** *Jules Remy* 386, Isl. Maui, 1851-1855 (type, Herb. Gray: cotype, Herb. Par.).

*PHYLLOSTEGIA MACROPHYLLA phytolaccoides* var. nov.—Inflor-  
escentia angustior elongatiorque fructifera tantum circ. 1.3-1.7 cm.  
crassa, verticillastris 6-floris.

**Specimens examined:** *A. S. Hitchcock* 14897, alt. 4000 ft., in very  
wet forest along pipe line, east of Olinda, East Maui, Oct. 1, 1916  
(type, Herb. U.S. Nat.).

*PHYLLOSTEGIA MACROPHYLLA velutina* var. nov.—Valde sericeo-  
villiosa vel demum velutina calysis circ. 6-7 mm. longi lobis acutis  
2-3 mm. longis, corollae tubo 8-12 mm. longo.

**Specimens examined:** *U.S.S. Pacif. Explor. Exped. under Capt.*  
*Wilkes*, Mauna Kea, Isl. Hawaii, 1840 (type, Herb. U.S. Nat.).

*Phyllostegia lantanoides* sp. nov.—Suffrutescens,  $\pm 4$  dm. alta,  
caule ramisque minutissime glandulosis nodis perspicue retrorsum-  
que nitido-setosis aliter nunc brevissime pubescentibus nunc glabra-  
tis. Folia opposita indivisa petiolo perspicue nitido-ciliato 1-2.8 cm.  
longo, lamina membranacea oblongo-ovata basi truncata vel sub-  
cordata apice acuta vel vix acuminata margine crenato-serrata  
utrinque minutissime numerosissimeque resinoso-glandulosa supra  
adpresso-hispidula infra non nisi ad venas hispidula 3-6.5 cm. longa  
et 1.7-3 cm. lata, interdum rugosa. Inflorescentia simplex spicato-  
racemosa, verticillastris paucis (circ. 4-6) 4- vel 6-floris, bracteis imis  
foliaceis  $\pm 2.5$  cm. longis aliis gradatim minoribus ovatis subsessili-  
bus basim versus albo-ciliatis. Calyx obconicus  $\pm 4.5$  mm. longus  
resinoso-glandulosus minutissime pubescens subacriter dentatus  
pedicella  $\pm 1.5$  mm. longa. Corolla alba vel punicea extrinsecus  
glanduloso-pubescentis circ. 1 cm. longa labio superiore quam inferiore  
circ. 0.6-0.65 brevior, filamentis pubescentibus, achaeniis triangu-  
latis obovatis apice subtruncatis circ. 2-2.3 mm. longis.

**Specimens examined:** *Anon.*, Hawaiian Isls. (Herb. Kew); *H.*  
*Mann* and *W. T. Brigham*, Kaala Mts., Isl. Oahu (type, Herb.  
Cornell Univ.).

*Phyllostegia bracteata* sp. nov.—Subherbacea scandens omnino  
molliter pubescens caulis ramorumque pilis plerumque retrorsis.

Folia opposita simplicia petiolo tenui 2-4 cm. longo lamina tenera ovata basi truncata vel subcordata apice subacuta margine crenata utrinque resinoso-glandulosa usque ad 5.5 cm. longa et 3.8 cm. lata. Inflorescentia simplex elongate racemoso-spicata 1.5-2.2 cm. longa, verticillastris saepius 8-12, remotis et plerumque 6-floris, bracteis imis foliaceis sub 3 cm. longis aliis rotundato-ovatis stipitatis calycem superantibus. Calyx breviter pedicellatus pedicella sub 2 mm. obconicus vel demum hemisphaericus circ. 3.5 mm. longus apud pilos resinoso-glandulosus acriter dentatus dentibus sub 1 mm. Corolla parva circ. 9 mm. longa extrinsecus pubescens, labio inferiore paulo longiore, filamentis fere glabratis, acheniis obovatis plano-convexis alatis atris 1.7-2.4 mm. longis.

**Specimens examined:** *A. S. Hitchcock* 14746, alt. 4000-5000 ft., vine in upper forest, Puu Kukui, West Maui, Sept. 24-26, 1916 (Herb. U.S. Nat., type); *H. Mann* and *W. T. Brigham* 415, West Maui (Herb. Gray; Herb. Mo. Bot. Gard.).

*Phyllostegia helleri* sp. nov.—Forsitan erecta. Folia ovato-cordata, breviter acuminata, crenato-serrata, laminis pilosis 8-10 cm. longis et 4-6.5 cm. latis, petiolis pilosis 2.5-4 cm. longis. Verticillastra 6-flora in racemis ramosis disposita, bracteis ovatis 3-4 mm. longis. Calyces glanduloso-pubescentes floriferi obconico-campanulati 4-5 mm. longi lobis subacutis circ. 1 mm. longis, fructiferi globoso-obconici, pedicellis subaequalibus. Corollae albae, pubescentes, circ. 1.2 cm. longae, tubo circ. 6-7 mm. longo.

**Specimens examined:** *A. A. Heller* 2875, on Kaholuamanoa, above Waimea, Isl. Kauai, Oct. 12, 1895 (type, Herb. U.S. Nat.: cotypes, Herb. Field Mus.; Herb. N.Y. Bot. Gard.); *A. S. Hitchcock* 15367, alt. 3600 ft., Kaholuamano, Isl. Kauai, Oct. 20, 1916 (Herb. U.S. Nat.).

*PHYLLOSTEGIA PARVIFLORA* var. *honolulensis* (Wawra) comb. nov.—*Phyllostegia honolulensis* Wawra, *Flora* 55:531. 1872; *ibid.* 58:286. 1875.

*PHYLLOSTEGIA BREVIDENS pubescens* var. nov.—A specie calycibus pedicellis foliorumque faciebus inferioribus dense pubescentibus caulibus moderate pilosis corollis minoribus 1.6-1.9 non 2.5-3 cm. longis lobis calycinis acutis differt.

**Specimens examined:** *Rev. J. M. Lydgate*, Upper Kula, Isl. Maui (type, Herb. Berl.).

*PHYLLOSTEGIA BREVIDENS pauciflora* var. nov.—A specie foliis (utrinque) et caulibus pubescentibus verticillastris 6-floris pedicellis (paulo longioribus, saepius 1.4–1.8 cm.) calycibusque pubescentibus lobis calycinis acutis differt; corollis ignotis.

**Specimens examined:** *Dr. William Hillebrand*, South Haleakala, Isl. Maui, September, 1870 (type, Herb. Berl.).

*PHYLLOSTEGIA BREVIDENS expansa* var. nov.—Folia demum sparsissime caules sparsim pilosa, verticillastris 6-floris, calycibus pedicellis pubescentibus lobis calycinis fere subulatis, corollis circ. 3 cm. longis extrinsecus manifeste pubescentibus labio plus expanso 1.5–1.8 cm. longo lobo mediano emarginato.

**Specimens examined:** *Dr. William Hillebrand*, Puu Kukui, West Maui (type, Herb. Berl.).

*Phyllostegia rockii* sp. nov.—*Phyllostegia hispida* var.  $\beta$ . Hillebr. Fl. Haw. Isls. 353. 1888.—Named for JOSEPH F. ROCK, who collected material of this form at Ukulele, Haleakala, East Maui and proposed it (*in* Herb. Berl.) as a distinct species.

*Phyllostegia wawrana* sp. nov. (sect. *Lateriflorae* A. Gray).—Omnino hirsuta, pilis mollibus patentibusque; forsitan herbacea. Folia magna inflorescentiam laminae longitudine superantia, longe petiolata petiolis usque ad 1 dm. longis, lamina membranaceissima suboblongo-ovata subcrenato-serrata serraturis ad unicum latus  $\pm 45$  basi subcordata apice breviter acuminata usque ad 2 dm. longa et 1.1 dm. lata. Racemi axillares, decompositi (3-partiti), verticillastris numerosis plerumque 6-floris; bracteis ovatis  $\pm 4$  mm. longis; pedicellis circ. 8–9 mm. longis; calycibus ad anthesin obconicis et circ. 5 mm. longis fructiferis subglobosis et circ. 4–5 mm. crassis lobis acutis 1–2 mm. longis; corollis sub 1 cm. longis labio superiore brevissimo (circ. 2 mm. longo).

**Specimens examined:** *Dr. Heinrich Wawra* 2060a, Isl. Kauai, 1868–1871 (2 type sheets, Herb. Mus. Vienna).

*STENOGYNE KAMEHAMEHAE albiflora* var. nov.—A specie corollis albis vel flavido-albis (nec vel aegerrime coccineo-purpurascens) differt.

**Specimens examined:** *A. S. Hitchcock* 14850, alt. 3000–5000 ft.,

Puu Kukui, West Maui, Sept. 24-26, 1916 (Herb. U.S. Nat.; *forma nonnullis corollis aegre subpurpurascens*); *idem* 14894, alt. 4000 ft., very wet forest along pipe line, east of Olinda, East Maui, Oct. 1, 1916 (Herb. U.S. Nat.); *Joseph F. Rock*, Honomanu, East Maui, May, 1911 (Herb. Gray); *idem*, Honomanui Gulch, East Maui, May, 1911 (Herb. Gray); *idem* 8549, trailing over a tree, Pohakumoa Gulch, halfway between Waikamoi and Honomanu Forest of Hamakua, East Maui, September, 1910 (type, Herb. Gray).

Here clearly belongs *Phyllostegia longiflora* Caum (Occas. Paps. Bish. Mus. 9: (5), 9, pl. 6. 1930), based on *Lyon* and *Caum* 150, a lone plant found along the Kula Pipe Line Trail in East Maui, and "noticeable by its conspicuous large white flowers." *Stenogyne longiflora* Dr. del Cast. (Illustr. Fl. Ins. Mar. Pacif. 57, tab. XXVII. 1886), based on *Remy* 379 from Maui, may likewise belong here, rather than with *S. kamehamehae* proper, although *DRAKE DEL CASTILLO*'s failure to describe the corolla color leaves the identity in doubt. His type seems no longer extant and his plate, uncolored as it is, affords of course no aid as to the corolla-color character.

*STENOGYNE SCROPHULARIOIDES skottsbergii* var. nov.—Folia saepius oblongo-lanceolata rarius ovata, calycis lobis acutis.

**Specimens examined:** *Charles Gaudichaud*, Hawaiian Isls. (type, Herb. Berl.); *Archibald Menzies*, Hawaiian Isls. (Herb. Kew).

Named for *DR. CARL SKOTTSBERG*, who had studied the type in 1933 and referred it to *S. scrophularioides* Benth. *BENTHAM*'s original description (Bot. Reg. 15: no. 1292, sub *Tribo* 6. Prasieae. 1830), however, was based on *Macrae*'s plant, with obtuse calyx-lobes.

*STENOGYNE SCROPHULARIOIDES remyi* var. nov.—Folia majora, petiolis sparsissime hispidis 1-1.5 cm. longis, laminis subglabris 5-6 cm. longis et 2.5-3.3 cm. latis, dentibus acrioribus breviter mucronulatis. Verticillastra 6-flora, calycibus acerrime lobatis circ. 7 mm. longis corollae circ. 2.5 cm. longis infra glabratis supra pubescentibus, pedicellis fructiferis 6-12 mm. longis.

**Specimens examined:** *Jules Remy* 376, Isl. Hawaii (type, Herb. Gray).

*STENOGYNE MACRANTHA gracilis* var. nov.—Suffruticosa, scandens, ramis elongatis gracilibus glabratibus, internodiis saepe 8-10 cm. longis. Folia oblonga vel ovato-oblonga, basi truncato-rotundata apice breviter acuminata petiolis 1-2.5 cm. longis laminis 6-7.5 cm.

longis et 2.7-4 cm. latis membranaceis glabratis pallidis. Verticillastria 6- vel interdum 4-flora, pedicellis fructiferis 1-1.5 cm. longis, calycibus campanulatis glabratis acriter irregulariterque lobatis (lobis 2-5 mm. longis inclusis) 6-7 rarius usque ad 9 mm. longis, corollis (siccis) flavido-albis infra forsitan aegre coloratis extrinsecus valde sericeis gutture valde ampliatis circ. 2 cm. longis labio inferiore quam superiore multo brevior, filamentis pilosis.

**Specimens examined:** *Joseph F. Rock* 10037, Hawaiian Isls. (2 type sheets, Herb. Gray).

*STENOGYNE MACRANTHA* var. *GRACILIS hispida* f. nov.—Caules ramique plus minusve retrorso-hispidi, internodiis paulo brevioribus, foliis viridibus saepe subrugosis infra saepe venosioribus. Calyces fructiferi 1-1.2 cm. longi pedicellis fere aequales. Corollae angustiores, 2.5-3 cm. longae.

**Specimens examined:** *Joseph F. Rock* 3511, Puulaalaa, west slope of Hualalai, Isl. Hawaii, June 10, 1909 (type, Herb. Gray); *idem* 3513, *eodem loco* (Herb. Gray).

*STENOGYNE ANGUSTIFOLIA* var. *hillebrandii* nom. nov.—*Stenogyne angustifolia* var.  $\gamma$ . Hillebr. Fl. Haw. Isls. 357. 1888.

*STENOGYNE ANGUSTIFOLIA* var. *mauiensis* nom. nov.—*Stenogyne angustifolia* var.  $\beta$ . Hillebr. loc. cit.

*STENOGYNE ANGUSTIFOLIA* *spathulata* var. nov.—Folia spathulata, apice subobtusum margine subvaldius serratum, petiolo adjecto 3-4.5 cm. longa et 7-9 mm. lata. Calyces minores 8-10 mm. longi, ad anthesin quam corollae circ. 0.65 (non circ. 0.5) breviores, bracteolis minutis  $\pm$  3 mm. longis.

**Specimens examined:** *Jules Remy* 393, Isl. Hawaii, 1851-1855 (type, Herb. Gray).

*STENOGYNE ANGUSTIFOLIA* *salicifolia* var. nov.—Folia lineari-lanceolata, apice sensim basi sensim vel subabrupte angustata margine subobscure serrata, petiolo adjecto 7-8 cm. longa et 0.9-1.2 cm. lata. Calyces  $\pm$  1.5 cm. longi, quam corollae circ. 0.4 breviores, bracteolis perspicuis circ. 1 cm. longis.

**Specimens examined:** *Dr. William Hillebrand*, Isl. Hawaii (type, Herb. U.S. Nat.).

*STENOGYNE RUGOSA* *mollis* var. nov.—Pube brevi molli induta, ramis saepe elongatis nodis remotis, verticillastriis 6-10-floris.

**Specimens examined:**<sup>2</sup> *Dr. William Hillebrand* 347, Isl. Hawaii (Herb. Gray); *H. Mann* and *W. T. Brigham* 295, woods at base of Mauna Kea, Isl. Hawaii (Herb. Gray); *United States South Pacific Exploring Expedition under Capt. Wilkes*, District of Waimea, Isl. Hawaii, 1840 (type, Herb. U.S. Nat.: cotype, Herb. Gray).

ASA GRAY (Proc. Amer. Acad. 5:348. 1862) presented a disposition of three sets of variants of *Stenogyne rugosa* Benth., without attempting the use of conventional varietal names. My recent study of a considerable amount of *S. rugosa* material showed the species proper to be highly polymorphic and to consist of the first two sets of variants described by GRAY. His third set, however, is very distinct varietally and well deserves the recognition here accorded it.<sup>3</sup>

**STENOGYNE PURPUREA leptophylla** var. nov.—*Folia* foliis lanceolatis vel anguste oblongo-lanceolatis (nec ovato-lanceolatis) plerumque sub 7 cm. longis et sub 1.8 cm. latis differt.

**Specimens examined:** *Joseph F. Rock* 5744, Hawaiian Isls. (Herb. Gray); *idem* 8863, Keaku stream, high plateau of Isl. Kauai, Oct. 21, 1911 (type, Herb. Gray).

**STENOGYNE RUGOSA subulata** var. nov.—*Folia* laminis sub 6 cm. longa et 2 cm. lata. Calycis lobi acerrime subulati, tubo longiores.

**Specimens examined:** *Dr. William Hillebrand*, between Kilauea and Kapapala, Isl. Hawaii (type, Herb. Berl.).

**Stenogyne calycosa** sp. nov.—*Stenogyne sessilis* var.  $\gamma$ . Hillebr. Fl. Haw. Isls. 359. 1888.

**STENOGYNE SESSILIS hexantha** var. nov.—*Folia* plerumque breviter petiolata petiolis 2–5 mm. longis. Verticillastra principalia 6–superiora 2–4-flora; calycibus ad anthesin circ. 8 mm. longis, lobis acutis; corollis circ. 2.2 cm. longis.

**Specimens examined:** *Joseph F. Rock* 10036, Hawaiian Isls. (2 type sheets, Herb. Gray).

**STENOGYNE SESSILIS** var. *lanaiensis* nom. nov.—*Stenogyne sessilis* var.  $\beta$ . Hillebr. Fl. Haw. Isls. 359. 1888.

**Stenogyne sororia** sp. nov.—Omnino glaberrima (corollis exclusis), foliis tenuiter petiolatis petiolo sub 1.5 cm. longo lamina simplici membranacea ovata basi subcordata apice breviter acuminata margine remote aegreque denticulata circ. 3.5–4.5 cm. longa.

<sup>2</sup> Only a small proportion of those studied are included here.

<sup>3</sup> HILLEBRAND's var. and  $\gamma$  (Fl. Haw. Isls. 356. 1888) is identical with this variety.

Verticillastra 6-flora, pedicellis saepius 7-9 mm. longis, calycibus obconicis (dentibus magnis acerrimis adjectis) 1.2-1.4 cm. longis; corollis immaturis extrinsecus pubescentibus.—An ally or sister species of *S. scrophularioides* Benth.

**Specimens examined:** *Dr. William Hillebrand*, Hawaiian Isls. (type, Herb. Kew).

**Stenogyne scandens** sp. nov.—Subherbacea, scandens vel procumbens, caule ramisque subglabris. Folia petiolata petiolis dorsaliter pubescentibus 5-10 mm. longis, lamina simplici membranacea saepius oblonga rarius lanceolato-oblonga basi truncata vel late cuneata apice obtusa vel rarius subacuta margine plerumque crenulato-dentata raro subacriter dentata supra venis minute hispidula aliter glabra infra praecipue ad venas irregulariter pilosa 2.5-3.5 (rarius -5) cm. longa. Verticillastra 6-flora, pedicellis sparsim pilosis tantum 2-5 mm. longis, calycibus late obconicis sparsissime setosis (lobis lanceolato-linearibus acribus adjectis) 6-8 mm. longis; corollis pallide purpureis extrinsecus valde pubescentibus,  $\pm$  1.8 cm. longis, labio inferiore brevissimo, filamentis (unico latere pubescentibus) styloque longe exsertis.—Habitu *Phyllostegiae racemosae* Benth. et *S. vaganti* Hillebr. similis.

**Specimens examined:** *Dr. William Hillebrand* 352, Isl. Hawaii (type, Herb. Kew: a probable duplicate, Herb. Berl.).

**Bidens hivoana** Degener & Sherff, sp. nov.—Frutex ramosus, glaber,  $\pm$  2 m. altus. Folia opposita, subconferte ad ramuli finem disposita, petiolata petiolis conduplicatis marginatis basi dilatatoconnatis 1.5-3 cm. longis, petiolo adjecto circ. 6-8 cm. longa et 2.5-3.8 cm. lata, indivisa, ovata, basi rotundata vel raro vix subcordata, apice subacuta vel subattenuata, membranacea, obsolete ac remote serrulata, eciliata. Capitula terminaliter circ. 3-adgregata, pedunculata pedunculis suberectis glabris  $\pm$  2.5 cm. longis, ut videtur radiata (ligulis in typo non plene cretis), disco ad anthesin circ. 6-7 mm. crasso et circ. 9-11 mm. alta. Involucri glabrati bractee exteriores circ. 4, ovato-oblongae vel late lanceolatae, obtusae, usque ad 8 mm. longae, quam interiores oblongae paulo longiores. Flores ligulati (fide lectorum descriptionis) albi. Paleae angustissime lineares, usque ad 11 mm. longae. Achaenia submatura plana, oblanceolata vel obovata, atro-brunnea, glabra, apice bidentata dentibus glaberrimis deorsum in margines membranaceas luteo-brunneas alis



similes desinentibus, corpore  $\pm 5.5$  mm. longa et marginibus alatis adjectis 2.3–3 mm. lata.

**Specimens examined:** *Adamson* and *Mumford* 469, growing 2 m. tall, alt. 3620 ft., in typical forest of cloud zone, on crest north of summit of Mt. Temetiu, Tenatinaei, Isl. Hiva Oa, Marquis Isls., Jul. 24, 1929 (type, Herb. N.Y. Bot. Gard.).

Recently Mr. OTTO DEGENER noted two specimens among the newer accessions in the Herbarium of the New York Botanical Garden. These he suspected of being new and had them forwarded to me for study. Each is found to be the type of a new species, bringing the total number of *Bidens* species indigenous to the Marquis Islands up to six. Mr. DEGENER courteously permits the use of his name in joint authorship.

***Bidens collina*** Degener & Sherff, sp. nov.—Frutex erectus, gracilis, ramosus ramis obscurissime adpresso-setosis circa 1 m. altus. Folia opposita, tenuiter petiolata petiolis saepius 1.5–2 cm. longis, petiolo adjecto 5–6 cm. longa et 1.5–3 cm. lata, indivisa, oblongo-lanceolata vel oblongo-ovata, rotundato-truncata vel fere subcordata, apice abrupte attenuata, membranacea, faciebus glabrata, marginibus acriter serrulata (unico latere 8–20-dentata). Capitula corymbose disposita, radiata, pansa ad anthesin 1.3–2 cm. lata et circ. 5 mm. alta. Involucri bractae hispidae exteriores 5–8, lineares vel oblongae apicem abrupte mucronulatum versus saepe dilatatae, 1.5–3 mm. longae, interioribus lanceolato-oblongis dimidio breviores. Flores ligulati plerumque 5 vel 6, flavi, ligula oblongi vel late oblanceolati, apice 2–3-denticulati, circ. 7–9 mm. longi. Achaenia submatura plana, lineari-oblonga, sursum sensim angustata, faciebus marginibusque perspicue erecto-setosa setis fulvescentibus corpore sub 2.5 mm. longa et sub 0.8 mm. lata, apice erecte setosa setis pluribus (saepe 2 longioribus et aristis non dissimilibus).

**Specimens examined:** *Adamson* and *Mumford* 400, growing about 1 m. tall on exposed hillside, alt. about 100 m., Tehutu, Isl. Hiva Oa, Marquis Isls., May 19, 1929 (type, Herb. N.Y. Bot. Gard.).

Described by the collectors as rare and unknown to many residents of Hiva Oa.

***Bidens onisciformis*** sp. nov.—Herba, unico ramo viso gracili subtereti sulcolato sparsissime piloso internodiis folia superantibus.

Folia opposita tenuiter petiolata petiolo  $\pm 5$  mm. longo adjecto sub 3 cm. longa, hispida setis pluriloculatis, bipinnatisecta segmentis ultimis membranaceis plus minusve oblongis acriter apiculatis vel dentatis dentibus vix mucronatis. Capitula pauca, tenuiter pedunculata pedunculis superne hispidis  $\pm 3$  cm. longis, ad anthesin cernua circ. 2 cm. lata et circ. 6 mm. alta, disco parvo demum circ. 6–7 mm. crasso. Involucri hispidi bracteae exteriores circ. 8, patentes, lineares,  $\pm 5$  mm. longae, quam interiores ovatae saepe paulo longiores. Flores ligulati 6 (pro unico capitulo ad anthesin viso), flavi, ligula elliptico-oblongeolati, apice vix denticulati, 8–10 mm. longi. Paleae lineari-oblongae, apice obtusae vel raro irregulariter mucronatae, circ. 5–5.5 mm. longae. Achaenia exteriora fertilia, obcompressa, moderate vel late oblonga, nigra, dorso  $\pm 8$ -sulculata et glabrata, marginibus pectinato-dentatis vel laceratis (non vere alatis) sursum albido-setosa, ventro circ. 8-sulculata et valde papillato-setosa setis albis, apice bidenticulata (sed vix aristulata) et erecte setosa, 4–5 mm. longa et 1.5–1.8 mm. lata; interiora sterilia, planiora, elongatiora, angustiora, griseo-nigra.

**Specimens examined:** *Van den Houdt* 211, alt. about 2200 m., northeastern Belgian Congo, 1932 (type, Herb. Brussels).

The outer achenes offer a strong superficial resemblance to sow bugs, of the genus *Oniscus*, whence the trivial name.<sup>4</sup>

***Bidens kivuensis* sp. nov.**—Herba parce suffruticosa, verisimiliter perennis,  $\pm 2$  m. alta, erecte ramosa, caule ramisque subtetragonis sparsissime hispidis setis articulatis. Folia opposita petiolata petiolis tenuibus  $\pm 1.5$  cm. longis hispidis setis patentibus pluriloculatis, petiolo adjecto 6–9 cm. longa, 2–3-pinnatisecta, segmentis ultimis lineari- vel lanceolato-oblongis membranaceis supra adpresse infra saepius patente hispidis acriter dentatis dentibus mucronatis sed non in setas productis. Capitula corymbose ad ramorum fines 3–5-adgregata pedicellis tenuibus pilosis setis patentibus subalbis pluriloculatis, radiata, pansa ad anthesin  $\pm 4.5$  cm. lata et  $\pm 8$  mm. alta. Involucri albido-hispidi bracteae exteriores circ. 8, oblongo-lineares, mediane 1-nervatae nervo atro, apice acutae, circ. 4 mm. longae, quam interiores oblongo-ovatae moderate longiores. Flores

<sup>4</sup> I have taken the name *Oniscus* in its restricted sense as used in modern Latin by zoölogists, rather than from the Greek.

ligulati plerumque 8, flavi, ligula oblanceolate lineari-oblongi, apice integri,  $\pm 2.2$  cm. longi. Paleae lineari-oblongae, circ. 5–6 mm. longae. Flores tubulosi tubo (æ corollae lobis aurantiacis) sparsim patenti-pilosi. Achaenia matura deficientia. Ovaria plana, lineari-oblonga, anguste marginata, faciebus marginibusque inferne glabrata superne erecto-setosa, apice erecte setosa et exaristata vel minutissime biaristata aristis glabris.

**Specimens examined:** *Jean Lebrun* 5467, a somewhat suffrutescent herb  $\pm 2$  m. high, on savanna at 1720 m. alt., Mulungu, Belgian Congo, May, 1932 (1st and 2nd type sheets, Herb. Bruss.: 3rd type sheet, Herb. Field Mus.).

Referred to *Bidens* with some degree of hesitancy in the absence of mature achenes. Should these prove to be definitely *alate*, a closer affinity with *Coreopsis* would be indicated. In *Bidens* the position occupied is close to *B. grantii* (Oliv.) Sherff, *B. gracilior* (O. Hoffm.) Sherff, *B. taylori* (S. L. Moore) Sherff, *B. elliotii* (S. L. Moore) Sherff, and *B. schlechteri* Sherff.

**BIDENS STEPIA *humbertii* var. nov.**—Gracilis,  $\pm 1$  m. alta, ramis erectis sparsim piloso-hispidis. Achaenia 6–7 mm. longa et circ. 1.7–1.9 mm. lata, exaristata, facie dorsali glabrata ventrali ac marginibus apiceque erecto-setosa.

**Specimens examined:** *H. Humbert* 7593, alt. 2400–2790 m., Biega Mountains, west of Lake Kivu, eastern Belgian Congo, March, 1929 (type, Herb. Bruss.).

**BIDENS GRANTII *scaettae* var. nov.**—Gracilis,  $\pm 5$  dm. alta, caule subsimplici glabrato vel supra sparsim piloso internodiis principalibus quam foliis multo longioribus. Folia breviter petiolata vel sessilia,  $\pm 5$  cm. longa, bi-tripinnatisecta, supra moderate infra densissime hispida, lobis lineari-oblongis ultimis acerrime apiculatis terminali 1.5–2 cm. longo et circ. 2–3 mm. lato. Achaenia corpore circ. 4 mm. longa et sub 1 mm. lata, aristis circ. 1.5 mm. longis.

**Specimens examined:** *H. Scaetta* 2286, Nyabihu, Ruanda District, easternmost Belgian Congo, 1930 (type, Herb. Bruss.); *idem* 2294, on clay soil in field unused for 10 years, dry country, *eodem loco et tempore* (Herb. Bruss.).

***Coreopsis curtisii* sp. nov.**—Herba erecta, verisimiliter perennis, gracilis, forsitan circ. 0.5 m. alta, subsimplex, caule angulato glaber-

rimo plus minusve purpureo-striato. Folia opposita, tenuiter petiolata petiolis glaberrimis basi connatis usque ad 2 cm. longis, petiolo adjecto usque ad 8 cm. longa, pinnata vel bipinnatisecta, laminae circumambitu triangulato-ovata, segmentis primariis pallidis membranaceis lanceolatis acriter dentatis secundum venas marginesque nunc sparsissime nunc subconferte albido-setulosi. Capitula pauca,  $\pm 3$ -adgregata et ad rami finem corymbose disposita pedunculis erectis tenuibus glaberrimis 4-8 cm. longis, radiata, pansa ad anthesin circ. 3-3.5 cm. lata et circ. 11-13 mm. alta. Involucri pallidi bractee supra reflexae, exteriores circ. 8 vel 9, lineari-oblongae, glabrae vel basi extrema hispidae, apice acutae,  $\pm 4$  mm. longae, interioribus ovato-oblongis apice glanduloso-pubescentibus moderate breviores. Flores ligulati circ. 8, aurantiaci, ligula anguste oblongi, apice integri vel subdenticulati,  $\pm 1.5$  cm. longi. Paleae lineari-oblongae, apicem versus valde aurantiacae, apice ipso subobtusae. Flores tubulosi extrinsecus glabrae vel secundum corollae superne aurantiacae dentes pubescentes et ad tubi apicem sparsim pilosi; stylorum ramis pulcherrime aurantiacis et elongato-appendiculatis. Achaenia matura non visa. Ovaria plana, oblanceolata, nitido-brunnea, longitudinaliter pluristriata, plus minusve alata, marginibus et faciebus saltem supra erecte setosa, corpore  $\pm 4$  mm. longa, apice valde erecto-setosa et biaristata aristis glaberrimis  $\pm 1.5$  mm. longis.

**Specimens examined:** *Mrs. Richard C. Curtis*, cultivated in 1930, from seeds obtained in Angola (type, Herb. Field Mus.).

A species offering a superficial resemblance in leaf outline and dissection to *C. oligoflora* Klatt. In the key proposed by me in an earlier article (BOT. GAZ. 91:314-317. 1931) for identification of the African species of *Coreopsis*, this species would run down to *C. monticola* (p. 316, line 10). The pertinent portion of that key may now be altered to read:

1. Involucrum non nisi basi hispidum

- m. Caulis rigidus superne circ. 2.5-3.5 mm. crassus, foliis principalibus petiolo adjecto circ. 4-5 cm. longis, capitulis pansis 4-5.5 cm. latis, planta kamerunensi. .... 10. *Coreopsis monticola*
- m. Caulis gracilis superne 1-2 mm. crassus, foliis principalibus petiolo adjecto 5-8 cm. longis, capitulis pansis 3-3.5 cm. latis, planta angolensi.

17a. *Coreopsis curtisii*

*Cosmos mattfeldii* sp. nov.—Herba perennis (Sectionis *Mesineniae*), gracillima,  $\pm 5$  dm. alta, caule subsimplici superne glabro inferne adpresso-hispido setis arcuatis retrorsis  $\pm 1.5$  mm. crasso e radice tuberosa. Folia opposita, petiolata petiolis tenuibus sparsissime hispidis et basi plus minusve hispido-ciliatis usque ad 2.5 cm. longis, petiolo adjecto saltem 5–8.5 cm. longa et 0.8–2 cm. lata, simplicia, lamina lanceolata vel ovato-lanceolata, membranacea, faciebus glabra, basi late apice anguste (vel angustissime) cuneata, marginibus minutissime spinuloso-ciliata et pauciter acridentata (unico latere plerumque 3- vel 4-dentata), inferiora quam internodia multo longiora superiora multo breviora. Capitula perpauca (tantum 2 pro unico specimine viso), longissime tenuissimeque pedunculata pedunculo 1.2–2.7 dm. longo et usque ad circ. 1 mm. crasso, radiata, pansa ad anthesin circ. 4.6 cm. lata et circ. 1.2 cm. alta. Involucri bractee exteriores circ. 8 vel 9, lanceolato-oblongae, glabrae, tergo circ. 5-nerviae, apice attenuatae vel acutae, circ. 8 mm. longae, interioribus ovato-oblongis apice glanduloso-pubescentibus paulo longiores. Flores ligulati 7 forsitan saepius 8, atro-sanguinei, ligula oblanceolato-oblongi, apice subintegri vel irregulariter dentati,  $\pm 2.3$  cm. longi. Paleae lineari-oblongae, glabrae, supra sanguineae, apice acutae et disci flores superantes. Flores tubulosi extrinsecus plerumque glaberrimi, superne sanguinei vel purpurascens. Achaenia matura non visa. Ovaria minuta, obcompressa, faciebus marginibusque glabra, apice biaristata aristis tenuibus retrorsum hamosis 5–6 mm. longis.

**Specimens examined:** *E. Langlassé* 369, in clay soil, alt. 900 m., La Laja, southern Mexico, Sept. 21, 1898 (type, Herb. Berl.).

Collected the same month and by the same collector as was *C. langlassei*, previously described by me (*cf.* Field Mus. Bot. Ser. 8:425. 1932). The locality, like many other of *Langlassé's* localities, is one absent from the atlases, but the states given on the *Langlassé* labels are Michoacan and Guerrero. The type, along with many other specimens of Compositae, had been obtained for my study from the vast accumulation of undetermined material at Berlin, by Dr. JOHANNES MATTFELD, after whom the species is gratefully named.

*Cosmos herzogii* sp. nov.—Herba suffruticosa verisimiliter peren-

nis, ramis gracilibus glabris sulculatis inferne subteretibus superne angulatis, caulium glabratorum internodiis inferioribus sub 1 cm. longis. Folia opposita, tenuissime petiolata petiolis 1-2 cm. longis, petiolo adjecto 5-7.5 cm. longa, glaberrima, circumambitu ovato-triangularata, bipinnatisecta, segmentis primariis plerumque 5 membranaceis eciliatis sparsim atro-punctulatis, ultimis lineari-oblongis 2-3 (-5) mm. latis apice acribus margine saepe 1- vel 2-dentatis dentibus acutis. Capitula perpauca vel solitaria ramos terminantia, radiata, pansa ad anthesin circ. 2.5 cm. lata. Involucri bractee exteriores circ. 8, lineari-attenuatae, glabrae, apice acutae, circ. 6 mm. longae, patentes; interiores lineari-oblongae, marginaliter diaphanae, apice minutissime pubescentes alibi glaberrimae, circ. 7-8 mm. longae. Flores ligulati circ. 8, flavi, ligula lineari-elliptici vel -oblongi, tantum circ. 6- vel 7-nervati, apice integri vel 2-dentati, circ. 1.3-1.5 cm. longi et 3-3.5 mm. lati. Paleae lineari-oblongae, marginibus integrae vel irregulariter acriterque 1-2-dentatae, circ. 7-9 mm. longae. Flores tubulosi extrinsecus sparsim adpresso-pubescentes; stylorum ramis extremo rotundatis abrupte longeque lineari-appendiculatis. Achaenia matura non visa. Ovaria plana vel subplana, cuneato-oblonga, nunc valde nunc aegre hispida, corpore circ. 1.5 mm. longa, apice 2- vel irregulariter 3-aristata aristis retrorsum 1-3-hamosis sub 0.5 mm. longis.

**Specimens examined:** *Theodor Herzog* 496, an abundant "half-shrub" ("Halbstrauch") on sandstone, alt. about 900 m., summit of Cerro San Miserate, Chiquitos, Department of Santa Cruz, Bolivia, May, 1907 (type, Herb. Berl.).

In leaf outline superficially resembling the purplish-rayed *C. peucedanifolius* of the Section *Discopoda*, but in characters of the capitula much closer to *C. langlassei* of the Section *Mesinenia*. Achenes and roots are much to be desired.

× *Dubautia fucosa* hybr. nov.—Rami (ramuli?) graciles, infra glabrati et moderate vel confertissime foliosi, supra hispiduli et subnudi. Folia opposita vel superiora alterna, plus minusve demissa, revoluta, linearia vel anguste lanceolato-linearia, basi subangustata marginataque, apice acriter attenuata, glaberrima, subcoriacea, apicem versus utroque latere minute 1-4-denticulata, manifeste 3- vel sub-5-nervia, 4.5-6.5 cm. longa et 3-5 mm. lata. Paniculae ramis

principalibus alternis et capitulorum notis *Dubautiae plantagineae* ipsi nisi pappo interdum paululum plumosiore valde similis. Achaenia fertilia.

**Specimens examined:** *Rev. J. M. Lydgate*, Isl. Lanai, Hawaiian Isls. (type, Herb. Berl.).

An undoubted hybrid. The inflorescence is that of *Dubautia plantaginea* proper, although the pappus is slightly more plumose than in that species and approaches that of *Railliardia*. The foliar habit, however, is quite unlike that of any *Dubautia* species but is much the same as that found regularly or at times in *Railliardia scabra* var. *leiophylla*, *R. coriacea*, *R. demissa*, and  $\times$ *Dubautia fallax*. The type was formerly in HILLEBRAND'S private herbarium and by him had been determined as *Railliardia molokaiensis* (for which cf. his citation of "Lanai," Fl. Haw. Isls. 226. 1888). From a consideration of geographic distribution, this hybrid would seem to be *Dubautia plantaginea  $\times$  *Railliardia scabra* var. *leiophylla*.*

$\times$  *Dubautia fallax* hybr. nov.—Rami hispidi, 2–4 dm. longi. Folia inferiora saepe confertissima demissaque superiora laxe disposita, lineari-lanceolata, apice acuta vel subattenuata, glaberrima, coriacea, utroque latere 1–6-denticulata, 3–8 cm. longa et 5–8 mm. lata. Inflorescentia *Railliardiae demissifoliae* et *R. thyrsiflorae* similis sed achaeniis sterilis et pappo prope *Dubautiam*.

**Specimens examined:** *Otto Degener* 4242, on rain- and fog-swept lava flow at top of Koolau Gap, within Haleakala Crater, Isl. Maui, Hawaiian Isls., Aug. 17, 1927 (Herb. Brit. Mus.; Herb. Field Mus.; Herb. N.Y. Bot. Gard.); *idem* 4273b, *eodem loco*, Aug. 11, 1927 (Herb. Berl.; Herb. Brit. Mus.; Herb. Deless.; Herb. Field Mus.; Herb. Gray; Herb. Mo. Bot. Gard.; Herb. N.Y. Bot. Gard.; Herb. Par.; Herb. U.S. Nat.; *cum Railliardia demissifolia* et *R. thyrsiflora commixta*); *Albert S. Hitchcock* 14955, alt. 6000–10000 ft., on rocky slope of Haleakala Crater, Isl. Maui, Oct. 2–5, 1916 (type, Herb. U.S. Nat.).

On comparison with the somewhat similarly leaved hybrid *Railliardia fucosa*, the inflorescence and habitat of which betray *Dubautia plantaginea* as indisputably one of the parents,  $\times$ *D. fallax* seems to be likewise a derivative from *D. plantaginea* in part or rather from a variety of *D. plantaginea*, since that species itself is known only from

Lanai. The shortly ciliate pappus bristles are, moreover, characteristic of *Dubautia*. In most characters of the inflorescence it is near to *R. demissifolia* and *R. thyrsiflora*, with which two species it was found by *Degener* to be growing intermixed.

**DUBAUTIA LAXA blakei** Degener & Sherff, var. nov.—Folia linearia vel anguste oblanceolata, apice acriter attenuata, acute sed pauciter spinuloso-denticulata, glabra sed saepe ciliata ac minutissime glandulosa plus minusve 3-5-(sub-7-) nervia. Involucri bractae extrinsecus glabratae vel sparsim hispidae.

**Specimens examined:** *Otto Degener* 4234, in windy rain forest, ridge north of Pohakea Gulch, Isl. Maui, Hawaiian Isls., Jul. 23, 1927 (3 type sheets, Herb. Field Mus.: cotypes, Herb. Berl.; Herb. Boiss.; Herb. Brit. Mus.; Herb. Univ. Calif.; Herb. Deless.; Herb. Gray; Herb. Kew; Herb. Mo. Bot. Gard.; Herb. Mus. Vienna; Herb. N.Y. Bot. Gard.; Herb. Par.; Herb. Phila.; Herb. U.S. Nat.; Herb. Univ. Vienna).

Named for Dr. SIDNEY F. BLAKE, who had made a preliminary study of various species of *Dubautia* and *Railliardia* and had recognized this as new.

**DUBAUTIA LAXA waianensis** Degener & Sherff, var. nov.—Folia oblonge obovata vel ovata, apicem obtusum vel abrupte acutum versus minutissime denticulata, utrinque subadpresse papillato-hispida, usque ad 5.5 cm. longa et 2.2 cm. lata. Inflorescentia corymbosa, laxior, pedicellis tenuibus pilosis suberectis saepe 1-2 cm. longis. Involucri bractae extrinsecus hispidae.

**Specimens examined:** *Otto Degener* and *K. K. Park* 4327, about 3-5 ft. tall, along summit ridge, between Puu Manawahua and Palikea, Isl. Oahu, Sept. 27, 1931 (Herb. Brit. Mus.; Herb. Deless.; Herb. Field Mus.; Herb. Gray; Herb. Kew; Herb. Mo. Bot. Gard.; Herb. N.Y. Bot. Gard.); *Kazuto Nitta* (*Otto Degener* distrib. no. 4340), alt. 2500 ft., in moderately wet locality, Mt. Kaala, Waianae Range, Isl. Oahu, Hawaiian Isls., 1929 (type, Herb. Field Mus.: cotype, Herb. N.Y. Bot. Gard.).

**DUBAUTIA KNUDSENII degeneri** var. nov.—Frutex  $\pm$  1.5 m. altus, ramulis hispidis. Folia obovata, sicca brunneo-viridia, distincte 5-nervia, subsparsim ciliata, inferne praecipue secundum venas plus minusve hispida.



**Specimens examined:** *Otto Degener* 4259, shrub 5 ft. high, in open rain forest, north of Pepeopae, northeastern Molokai, Hawaiian Isls., May 8, 1928 (type, Herb. Field Mus.); *idem* 4261, in rainy region, on crest of Waikolu Valley, Isl. Molokai, May 1, 1928 (Herb. Field Mus.; Herb. N.Y. Bot. Gard.).

***Dubautia paleata* × *D. waialealae* hybr. nov.**—Folia conferta internodiis hispidis saepius 2–6 mm. longis, spathulato-oblancoolata, nunc 4–5 cm. longa et 7–12 mm. lata nunc 7–9 cm. longa et 1.2–1.6 cm. lata, apice acuta basi 3–6 mm. lata, utrinque adpresso-hispida. Capitula paniculata vel compacte ac contracto-corymbose disposita, varia sed iis *Dubautiae waialealae* propria.

**Specimens examined:** *Albert S. Hitchcock* 15468, in bog, alt. 3600–5080 feet, Waialeale, Isl. Kauai, Hawaiian Isls., Oct. 22–24, 1916 (Herb. U.S. Nat.); *idem* 15492, *eodem loco et tempore* (Herb. U.S. Nat.).

Closer to *D. paleata* Gray in size, shape, texture, and arrangement of leaves, but closer to *D. waialealae* Rock in compactness of the inflorescence and most characters of the capitula. The indubitable occurrence of hybridity shown in this case between the bizarre *D. waialealae* and one of the more orthodox species of *Dubautia* is important in that it raises a query as to the integrity of *D. waialealae* var. *megaphylla* Sherff.<sup>5</sup>

× ***Railliardia dolosa* Degener & Sherff, hybr. nov.**—Folia opposita, patentia vel demissa, oblonge et interdum anguste lanceolata oblanceolatae, sessilia, rigida, apicem acutum versus obsolete serrulata, 5–7-nervia, faciebus glabrata, marginibus ciliata, principalia 3.5–5.5 cm. longa et circ. 1–1.4 cm. lata. Capitula laxa et racemoso-paniculate disposita pedicellis pilosis suberectis saepius 1–3 cm. longis, involucri obconico-campanulato 6–8 mm. alto. Hybrida, probabiliter inter *Railliardiam menziesii* et *R. platyphyllam*.

**Specimens examined:** *Otto Degener*, Koolau Gap, Haleakala Crater, Isl. Maui, Hawaiian Isls., Aug. 19, 1927 (Herb. Field Mus.); *idem* 4243, tree 10 ft. tall, in rain forest just below summit of Koolau Gap, Aug. 17, 1927 (Herb. Field Mus.; Herb. N.Y. Bot. Gard.); *idem*

<sup>5</sup> The leaves of *D. waialealae* var. *megaphylla* have the appearance of being diminutive, somewhat metamorphosed leaves of *D. plantaginea* Gaud. or one of its several varieties.

4270, sparingly branched shrub about 2-4 ft. tall, found especially on inner slopes of and on driest lava at bottom of Haleakala Crater, Aug. 20, 1927 (Herb. Field Mus.; Herb. N.Y. Bot. Gard.); *idem* 4271, *eodem loco*, near Kaupo Gap, Aug. 20, 1927 (3 type sheets, Herb. Field Mus.: cotypes, Herb. Berl.; Herb. Boiss.; Herb. Brit. Mus.; Herb. Univ. Calif.; Herb. Deless.; Herb. Gray; Herb. Kew; Herb. Mo. Bot. Gard.; Herb. Mus. Vienna; Herb. N.Y. Bot. Gard.; Herb. U.S. Nat.).

× *Railliardia vafra* Degener & Sherff, hybr. nov.—Frutex ± 9 dm. altus. Folia opposita, elliptico-oblonga vel lanceolato-oblonga, sessilia, apice subacuta, integra, subcoriacea, plerumque 5-nervia, glabra atque eciliata sed minutissime plus minusve glandulosa, 3-4 cm. longa et 6-10 mm. lata. Inflorescentia capitulorumque notis *Railliardiae linearis* non dissimilis. Hybrida, probabiliter inter *R. linearem* et *R. menziesii*.

**Specimens examined:** *Everett Brumaghim* (*Otto Degener* distrib. no. 4336), near Kilauea on Kau side, Isl. Hawaii, Hawaiian Isls., October, 1932 (Herb. Field Mus.; Herb. N.Y. Bot. Gard.); *Otto Degener* 2134, a shrub 3 ft. tall, 25 miles from Waimea toward Kona, Pahoehe Desert, Isl. Hawaii, Aug. 18, 1926 (2 type sheets, Herb. Field Mus.: cotypes, Herb. Berl.; Herb. Brit. Mus.; Herb. Gray; Herb. Kew; Herb. Mo. Bot. Gard.; Herb. N.Y. Bot. Gard.); *idem* 4226, *eodem loco et tempore* (Herb. Field Mus.; Herb. N.Y. Bot. Gard.).

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# COMPARATIVE MORPHOLOGY OF DUMORTIERA HIRSUTA

SISTER MARY ELLEN O'HANLON

(WITH THIRTY-FIVE FIGURES)

## Introduction

In 1919, EVANS (4) published the results of an investigation on the taxonomy of *Dumortiera*. Owing to the untrustworthy nature and inconstancy of certain differential characters which have been employed by previous writers to define the various species of *Dumortiera*, EVANS concluded that there are but two known species of this genus, *D. hirsuta* and *D. nepalensis*. He bases the specific differences on the general character of the upper surface of the thallus in each species.

The present study was undertaken chiefly to observe the process of spore germination and to compare it with that of some of its congeners, namely, *Marchantia polymorpha* (7), *M. domingensis* (10), *Preissia quadrata* (8), *Conocephalum conicum* (6), and *Reboulia hemisphaerica* (9). The plant material was furnished through the courtesy of Professor HERMAN KURZ, who collected it near Tallahassee, Florida.

CAMPBELL (2) states that *Dumortiera hirsuta* occurs in the British Isles, Japan, and in the southeastern United States. In his study of the East Indian hepatics, he makes reference to *D. trichocephala*. His description of the same species from the Hawaiian Islands is in full agreement with EVANS' key to *D. hirsuta* and also with the results of this study.

## Investigation

### CARPOCEPHALUM AND SPOROPHYTE

Plants bearing ripe sporophytes reached the laboratory on April 20. Many of the capsules had already begun dehiscence and some of the spores were sown immediately in small glass stender dishes filled with a mineral nutrient solution. These cultures were placed in north, south, and east exposures of the same room. Some of the

carpocephala and pieces of the thalli were killed in chrom-acetic acid solution for a study of the adult plant.

The carpocephalum is borne on a stalk which has two groups of rhizoids on the side which corresponds to the ventral side of the thallus. The archegonia are borne in groups of which eight is probably the average number. Usually one, sometimes two sporophytes come to maturity in each of the groups.

The sporophyte (fig. 1) is made up of the usual parts: foot, seta, and capsule, and seems to resemble in its general structure the sporophyte of *Marchantia* and its nearer relatives more than it does some of the other members of the family. The foot is indistinctly anchor-like; the seta is prominent, massive, and more or less columnar; the capsule is oval in its general contour, with a slight depression at the basal end. The whole is surrounded by a calyptra which is six cells in thickness. The spore output, estimated at 50,000 spores to a capsule, falls short of that of *Marchantia polymorpha* which, with its approximate 300,000 spores, probably reaches the peak of spore production in these hepatics; it exceeds *M. domingensis* with a capsular output of about 20,000 spores; and it still further surpasses in spore production *Preissia* (8), *Conocephalum* (6), and *Reboulia* (9), which with their increased proportionate numbers of elaters and greater spore size have much reduced numbers of spores. The elaters of *D. hirsuta* would be difficult to distinguish from those of *Marchantia* in form, size, and color, and their number in proportion to the spore number is probably about the same as in the case of *M. domingensis* (1), that is, 32 or 64 spores to each elater.

#### SPORE GERMINATION

The size of the spore of *D. hirsuta*, about 30  $\mu$  in its longest axis, is also nearer to that of *M. domingensis* than it is to any of the others with which comparison has been made. The spores, which were sown on a liquid medium, showed signs of germination on the sixth day. This was manifest by their increased size and their extended form. Germination was 100 per cent. The volume of a germinating spore is probably four or more times that of a newly shed spore (figs. 2, 3). In this feature, *Dumortiera* inclines to the condition in *M. polymorpha*, where there is great increase in the spore size prior to germina-

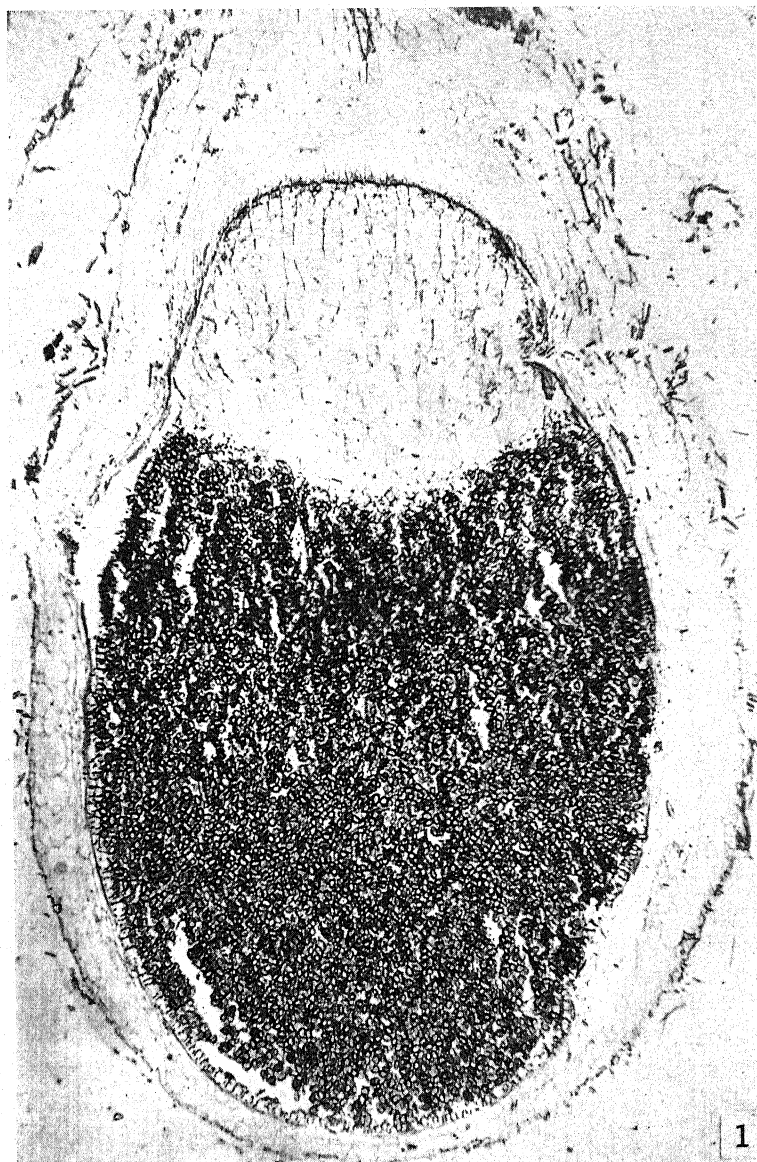


FIG. 1.\*—Median longitudinal section through mature sporophyte of *Dumortiera hirsuta*.

\* All drawings were made with the use of a camera lucida from living material and show uniform magnification of  $\times 130$ .

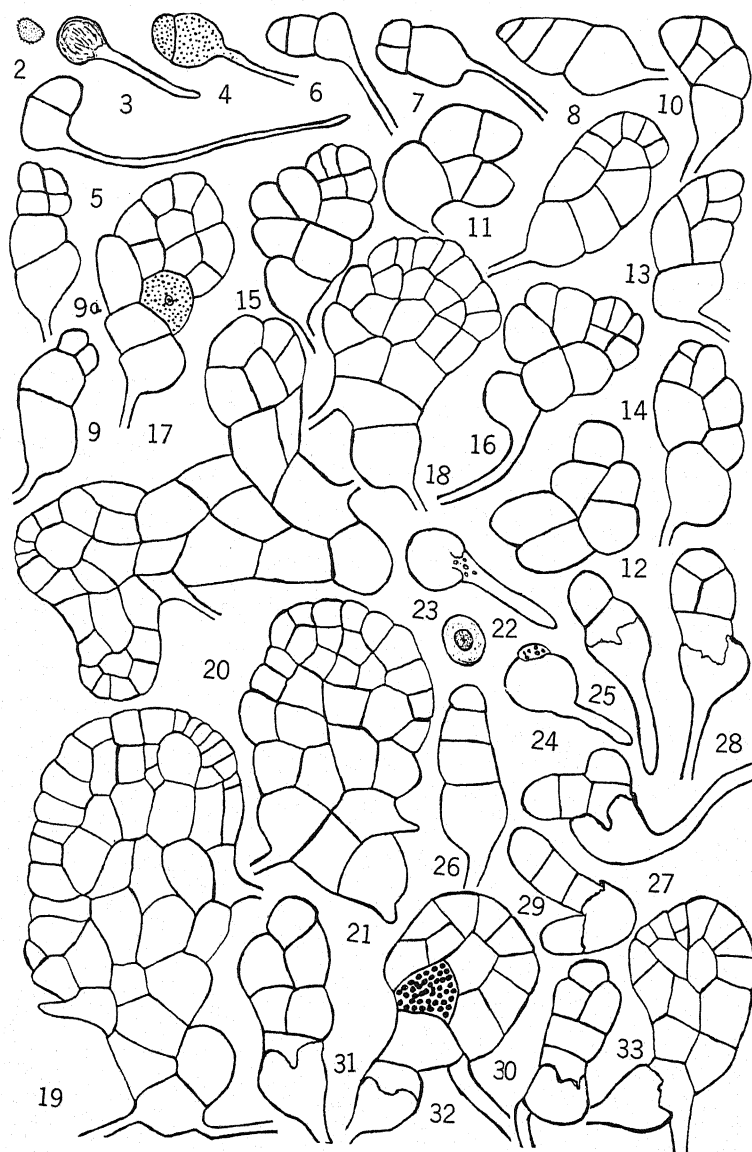
tion (7). In other species studied there was little or no distention of the spore before germination (8, 9, 10).

The first step in the germination process in the spore of *Dumortiera* is usually the emergence of a rhizoid, which is of particularly small caliber in this species, that is, in the sporelings and young gametophytes. The appearance of this primary rhizoid is followed by an unequal division of the spore cell. Cell division (or rather budding, inasmuch as each successive new cell is the result of an unequal division) continues until three or four cells in a linear series are formed (figs. 3-6, 8, 10). Then follow cell divisions at the tip of the filament, most often in an oblique plane, which give rise to an active, wedge-shaped, apical cell typical in the young developing gametophyte of the true ferns (figs. 13-16). This kind of cell division continues as the growth process advances, and it may occur in every cell of the original filament, the first cell only excepted (figs. 19-21). There are deviations from this more or less regular procedure, such as the early initiation of twin thalli (figs. 11, 20); or divisions in the second plane may occur early as in figures 7, 9, and 9a. Occasionally the primary rhizoid is much belated (fig. 21).

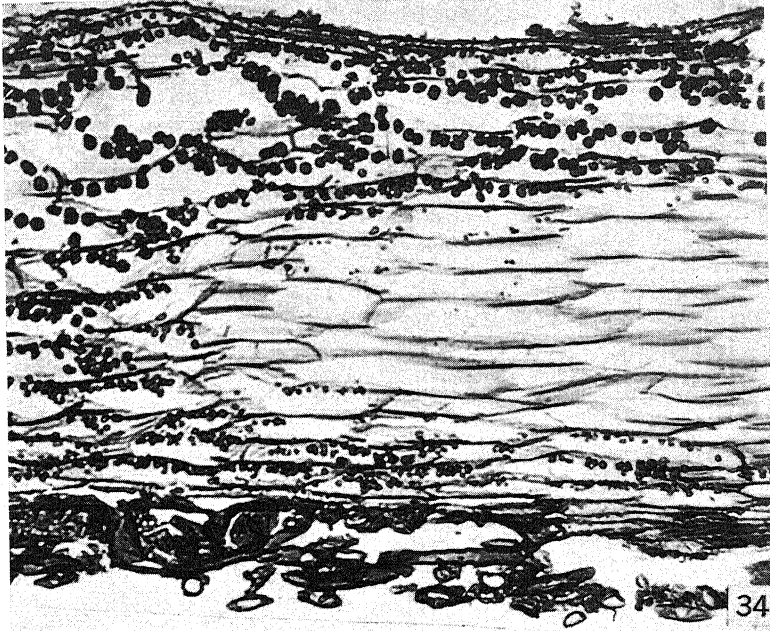
Besides the unusually small caliber of the rhizoids in the young gametophytes of *Dumortiera*, another distinctive feature is the exceptionally minute chloroplasts (fig. 17). In the adult thalli, however, the rhizoids seem to be as large as those of any of the other liverworts, and the chloroplasts are unusually large and plentiful (figs. 34, 35).

#### COMPARISON OF DUMORTIERA AND ONOCLEA STRUTHIOPTERIS

In tracing the development of the young thallus of *Dumortiera*, one is struck with the fernlike character of each figure. A comparison of the sporelings of *Dumortiera* with figures of *Onoclea struthiopteris* (2) showed the close resemblance between corresponding stages in the development of these two genera. Cultures of the spores of *Onoclea* were set up and typical stages were figured for comparison with those of *Dumortiera*. Unfortunately the cultures of these plants could not be grown simultaneously because spores of *Dumortiera* are viable for a few days only, after the dehiscence of the capsule, and the spores of *Onoclea* are not ripe until late autumn. There



FIGS. 2-33.—Figs. 2-6, 8, 10, 13-19, ripe spore and early stages in development of young gametophytes of *D. hirsuta*; figs. 7, 9-9a, 11, 12, 20, 21, young gametophytes of *D. hirsuta* which show irregularities in their development; figs. 22, 33, spore (with coat removed) and early stages in development of young prothallium of *Onoclea struthiopteris*.



FIGS. 34, 35.—Longitudinal and transverse sections through adult thallus of *Dumortiera hirsuta* showing non-chlorophyllose cells in thickest part of thallus.



was little difficulty, however, in matching practically all of the figures previously drawn of *Dumortiera* with the growing material of *Onoclea*. CAMPBELL considers *Onoclea* as typical of the Polypodiaceae, so that on this basis these results may be considered as representative for this group of ferns.

The regular procedure, including emergence of the primary rhizoid (fig. 23), transverse budding process followed by the oblique divisions, and early establishment of the cuneate apical cell (figs. 24-26, 28, 30, 31), is strikingly similar in *Dumortiera* and *Onoclea*. Even such irregularities as the introduction of twin thalli (fig. 27), the suppression of the primary rhizoid, and other early digressions (fig. 29) occur with about equal frequency in the two genera.

The chief morphological differences observed from the spores to the 13-20-celled stages in the gametophytes of these two species were: spore size, spore coats, diameter of the rhizoids, and number and size of the chloroplastids. The spore of *Onoclea* (fig. 22) is larger, increases relatively little before germination, and has a three-layered coat which is adherent even after the prothallium has assumed a definite heart-shape. The spore coat is early deciduous in *Dumortiera*. The chloroplasts in the young prothallium of *Onoclea* are several times as great in diameter as those of the young thalli of *Dumortiera* and are relatively fewer in number (figs. 17, 32).

Other differences noted in the two genera were more or less physiological and were beyond our control since the cultures of the two plants were not grown simultaneously. In general, the spores of *Onoclea* germinated more promptly, and more especially so in an atmosphere that was artificially heated, and the sporelings advanced more rapidly in north light than was the case in either the east or west exposures. The short daylight periods beginning November 4, the date on which the spores of *Onoclea* were sown, seemed to have no retarding effect; in fact, germination was equally prompt, if not more so, in cultures set up on December 10. Subsequent development of these young sporelings, however, was less than in the cultures started in November. Cultures placed in the south windows, one in a cool room and one in another kept at living-room temperature, gave negative results in the November sowings. Repetition of these tests in December showed germination in both cases, that in the warmer room being relatively much better. The optimum re-

sults of all of the spore germination experiments with *Onoclea* seemed to be in the warmer temperature (about 20° C.), on an average, in the north light exposure, and in (at least for development subsequent to germination) the longer daylight periods.

The conclusions drawn from these germination tests of the spores of the two genera are: that *Onoclea* can tolerate much less direct sunlight; needs at least moderate temperature; and seems to do well, probably best, in short daylight periods. *Dumortiera*, although also shade-loving, is more tolerant of direct sunlight and may need the longer daylight periods for its best expression in the matter of spore germination and subsequent development of the young gametophyte. At any rate, it seems that the season of long daylight is the only time at which spore germination could occur in *Dumortiera*, since its spores mature at this season and are ephemeral. It is likely too that a warmer temperature would have hastened germination in this exotic species. The spores of *Dumortiera* require about three weeks from the date of sowing to reach the 5-7-celled stage; those of *Onoclea* made similar progress in about half the time. Thus the differences observed in the germination process and in the development of the young gametophytes in *Dumortiera* and *Onoclea* are more physiological than morphological.

CAMPBELL (2) compares the early schedule of cell division, etc., in *Onoclea* with the same processes in *Aneura*, which represents the simplest of the liverworts, and says that the early development of the gametophyte of *Marattia*, a primitive fern, is also much the same. On this basis *Dumortiera* may be considered primitive, at least in its method of thallus development. The relative simplicity of the adult gametophyte (figs. 34, 35) is also consistent with this theory.

#### COMPARISON OF DUMORTIERA WITH SOME OF ITS CONGENERS

In the study of spore germination of six species of hepatics, *Marchantia polymorpha*, *M. domingensis*, *Conocephalum conicum*, *Preissia quadrata*, *Reboulia hemisphaerica*, and *Dumortiera hirsuta*, there seem to be four fairly distinct types, the *Marchantia-Preissia* type, the *Conocephalum* type, the *Reboulia* type, and the fern type which is represented by *Dumortiera*. The *Marchantia-Preissia* type bears considerable resemblance to the true ferns and therefore to *Dumortiera* in the early stages of the development of the gameto-

phyte, but shows much more irregularity in these early stages. And instead of the unequal division of the spore cell and the continuance of this budding process to form the short filament, these divisions in the *Marchantia-Preissia* type are more nearly if not entirely equal. Neither is it so easy to designate the apical cell in many of the young gametophytes of the *Marchantia-Preissia* type. MENGE (5), however, followed the complete development of individual sporelings of *Marchantia polymorpha* from the sowing of the spores to the fairly well advanced young gametophytes, and shows convincingly the activity of a 2-angled apical cell.

*Conocephalum* is probably unique among the Marchantiaceae with its intracapsular spore cell divisions up to the 30-40-celled stage before dehiscence of the capsule.

*Reboulia* initiates its thallus from a primordial group of cells which is invariably borne at the tip of a germ tube, whether this latter be of primary, secondary, or tertiary origin. MENGE presents results from the study of gametophyte development of *Plagiochasma* which bear marked resemblance to corresponding stages of spore germination and subsequent development of the young gametophyte of *Reboulia* (9). The adult thalli of these two genera are also remarkably similar in structure.

*Dumortiera* closely resembles the true ferns from spore germination to about the 18-20-celled stages, when the young prothallus of the fern begins to take on a definite heart-shape.

#### ADULT GAMETOPHYTES OF TYPICAL GENERA

An examination of the adult gametophyte of *Dumortiera* in both longitudinal and cross-sections (figs. 34, 35) shows the total absence of lacunae and the rather even distribution of the extraordinarily large chloroplasts throughout the tissues of the thallus, excepting in an area in the mid-region somewhat posterior to the tip (that is, in the thickest part of the thallus, there are no chloroplasts). Since chloroplasts are found ventral to this area, their absence in the middle portion of the thallus cannot be attributed to insufficient light. Perhaps these non-chlorophyllose cells are specialized to meet the greater conduction need in the thicker part of the plant.

*Dumortiera* has no ventral scales, despite the fact that these organs

are characteristic of the Marchantiaceae. CAMPBELL (2) says that there are none also in *Monoclea*, another aberrant genus which is closely allied to *Dumortiera*. The rhizoids, that is, those of the plain walled type, are of unusually large caliber in the adult thallus of *Dumortiera*, whereas those with pegged walls, although very numerous, are reduced in diameter.

### Summary

1. *Dumortiera hirsuta*, probably one of two species only of this genus, is widely distributed in the tropics of both hemispheres and also in the more humid and warmer regions of the temperate zones.

2. The carpocephalum is covered with hairlike appendages and is borne on a stalk which has two groups of rhizoids on the side which corresponds to the ventral side of the thallus, this feature being the only suggestion of dorsiventrality in the stalk.

3. One or two sporophytes in each of the average number of eight groups of archegonia come to maturity. The sporophyte is made up of a rather indistinctly anchor-like foot, a massive columnar seta, and an ovate capsule which is depressed at the basal end. The whole is incased in a calyptra which is six cells in thickness.

4. The spores are ripe in Florida about the middle of April. They are viable for but a short time after the dehiscence of the capsule. The ripe spore is somewhat trihedral in form, is about  $30\ \mu$  in its longest axis, and is surrounded by a golden brown papillate exine.

5. The elaters of *Dumortiera* resemble those of *Marchantia* in structure, size, and color; the proportionate number of spores and elaters agrees well with the condition in *Marchantia domingensis*.

6. The spores of *Dumortiera* germinate within six or seven days after being sown on a liquid medium. The first step in germination, following a considerable distention of the spore, is usually the emergence of a rhizoid followed by the formation of a bud from the spore.

7. The budding process most often continues until a short filament of three or four cells is formed. In the short filament of cells, divisions at the tip, usually in an oblique plane, result in the establishment of a wedge-shaped apical cell of the fern type. This zigzag type of cell production continues and may occur in every cell, the original cell only being excepted, as growth advances.

8. The young sporelings of *Dumortiera* are remarkably like those

of *Onoclea*, a typical Polypodiaceae, throughout the germination process and in the early stages of the development of the young gametophyte.

9. The adult thallus shows no lacunae and there is no definite line of demarcation between the chlorophyllose and the colorless cells, which latter are found in the more or less central part of the thallus; and there are no ventral scales on the thallus of *Dumortiera*.

10. *Dumortiera* may be considered an aberrant species because of the relative simplicity of the gametophyte; and the primitive features, such as the absence of ventral scales, lacunae, etc., are possibly only consistent with the history of the young gametophyte from the spore.

The writer is indebted to Dr. ROBERT G. GUTHRIE for the photomicrographic work for the illustrations in this paper.

ROSARY COLLEGE  
RIVER FOREST, ILLINOIS

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## INFLUENCE OF SUPPLEMENTAL LIGHT ON BLOOMING

FRANCIS RAMALEY

(WITH THREE FIGURES)

This paper describes a series of studies carried on during each academic year from 1927 through 1933 to determine the effect upon plants of lengthened daily light exposure. Two Mazda lamps of 100 to 200 watt were placed in a reflector above the greenhouse bench, giving, at the height of the plants, from 15 to 30 foot-candle illumination. Since the greenhouse is well ventilated these lamps did not raise the temperature of the plants an appreciable amount. As a rule, artificial illumination of 5 foot-candles or less produces no visible effect, while 100 foot-candle illumination has little more influence than the 15 to 30 foot-candle illumination employed in these experiments. The moderate light here used could well be afforded by florists or others who might wish to hasten blooming of plants for commercial purposes.

One hundred species were employed for the investigation. The plants were grown in 5-inch pots, from 10 to 100 plants of each species being used in each case, always in four or more pots. When past the early seedling stage those which were to be irradiated were given artificial illumination from 5:00 until 10:00 P.M. or even all night. It makes very little difference in the time required for flowering in winter whether the day length is increased by supplemental light to 15, 20, or 24 hours.

The control plants, shaded from the artificial light, were placed on the bench a short distance from the experimental plants and were thus subjected to the same temperature and humidity. The greenhouse is not provided with automatic heat control, but an attempt was made to keep the rooms between 60° and 80° F. Since the temperature cannot be controlled during the summer the experiments were carried on only during the cooler months.

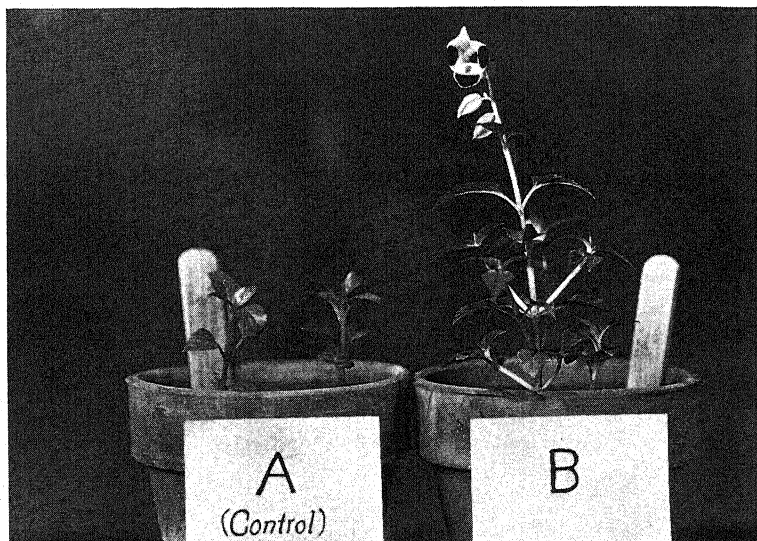


FIG. 1.—*Torenia fournieri*, a species hastened by supplemental light; planted Oct. 27, 1931: B, at right given supplemental light (30 foot-candles, continuous) from Feb. 26 to Apr. 7, when photographed.

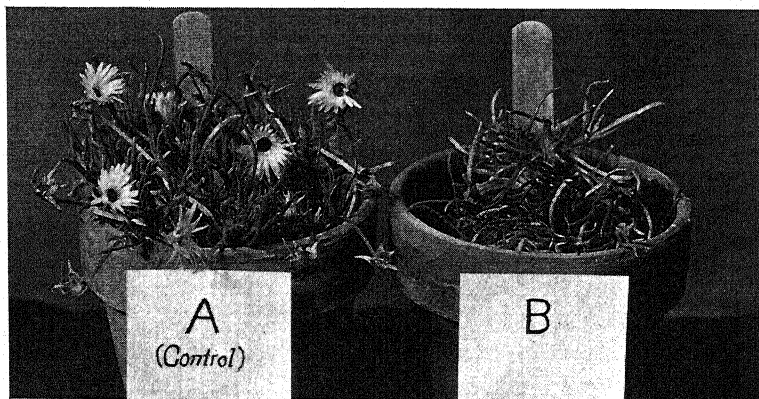


FIG. 2.—*Mesembryanthemum tricolor*, a species retarded by supplemental light; planted Oct. 31, 1931: B, at right, given supplemental light (30 foot-candles, continuous) from Dec. 5 to March 5, when photographed.

### Observations

In nearly all cases the experimental plants grow taller, with lengthened internodes; and they often have appreciably thinner leaves and a slightly paler color than the controls. These etiolation effects are naturally to be expected because of the weak artificial light to which the plants are exposed for a time each day. An under-development of root system commonly occurs, with reduced

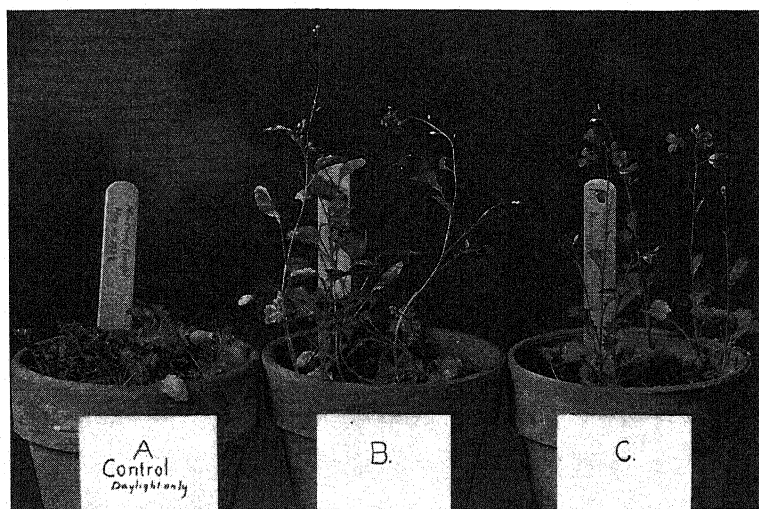


FIG. 3.—*Brassica alba*, planted Nov. 24, 1932; photographed Feb. 4, 1933: A, exposed only to daylight; B, exposed only to electric light (250 foot-candles, continuous); C, daylight plus supplemental light (30 foot-candles, continuous).

vascular tissue, especially phloem, while the stems tend to be lax. The leaves are not usually altered in size, but in a few instances are distinctly reduced. In radishes the hypocotyl elongates but does not swell to produce a "good radish." Red color of petioles and leaf midribs seldom develops even if present in the control series. No cases have been seen in which red or purple colors have appeared in the experimental plants when absent from the plants subjected to ordinary day length.

The most striking changes among the plants studied are in the time required until blossoming. Marked effects of increasing day



length appear in the winter months, while a few hours of extra illumination or even continuous 24-hour light has little influence in the long and hot days of summer.

As would be expected in view of the work of GARNER and ALLARD and many others,<sup>1</sup> some plants bloom quickly with lengthened illumination (long-day plants), some are delayed (short-day plants), while with others there is no marked difference between the experimental plants and the controls. Many plants have been tested in addition to those recorded in table I, and in none did the supplemental light affect to any extent either the vegetative or the reproductive activity.

Although exact dates of planting and blooming have been kept, it has seemed unnecessary to include them in the table, hence only the length of time required to bring the plants to blossom is given. The time of blooming is considered, not as the day on which the first single open flower is seen but the time when a considerable number of flowers have appeared. The localities named in the table are the native habitats, so that one may judge the length of day to which the species was originally exposed.

Certain species have been worked with during different years; in the table the results of the individual experiments are separately stated. These are often fairly consistent but sometimes are wide apart. Inconsistent results may be due to differences in time of year and in cultural care, or they may be of genetic origin, brought about by employing different horticultural varieties derived from diverse sources.

An asterisk in the table indicates that the plants did not bloom during the period of the experiment. The letters in the column following the names of plants have the following meanings: h, blooming hastened; r, blooming retarded; u, blooming unaffected.

<sup>1</sup> For further literature on the subject, see RAMALEY, F., A working bibliography of day length and artificial illumination as affecting growth of seed plants. Univ. Colorado Studies 20:257-263. 1933.

TABLE I  
TIME FROM SOWING OF SEEDS TO BLOOMING,  
STATED IN NUMBER OF DAYS

SPECIES AND LOCATION	BLOOM- ING RE- SPONSE	EXPERI- MENTAL PLANTS (DAYS)	CONTROL PLANTS (DAYS)
POACEAE			
Triticum aestivum var. Marquis (western Asia)	h	78	*
Zea mays (tropical America)	r	*	121
POLYGONACEAE			
Fagopyrum esculentum (central Asia)	u	70	70
CHENOPODIACEAE			
Atriplex hortensis (Eurasia)	u	91	91
Blitum capitatum (temperate North Africa)	h	93	171
AMARANTHACEAE			
Amaranthus sp.	r	163	71
"		141	89
Celosia argentea (west Asia)	u	137	137
NYCTAGINACEAE			
Abronia umbellata (British Columbia to California)	h	120	153
AIZOACEAE			
Mesembryanthemum lineare (South Africa)	r	184	119
CARYOPHYLLACEAE			
Dianthus barbatus (Eurasia)	h	80	138
Dianthus caryophyllus (so. Eurasia to India)	h	162	182
"		172	197
"		133	144
Dianthus plumarius (Eurasia)	h	128	154
Gypsophila paniculata (Eurasia)	h	55	75
"		48	83
"		98	180
Lychnis coeli-rosa (Mediterranean)	h	70	160
Lychnis viscaria (Eurasia)	h	62	*
Saponaria "multiflora"	h	54	100
Silene vulgaris (Eurasia)	h	120	*
RANUNCULACEAE			
Adonis aestivalis (central Europe)	h	152	166
"		115	135
Delphinium ajacis (south Europe)	h	91	135
Nigella damascena (south Europe)	h	79	119
PAPAVERACEAE			
Argemone sp. (southwestern United States)	h	102	150
Papaver rhoeas (Eurasia)	h	76	144
BRASSICACEAE			
Brassica alba (Eurasia)	h	48	90
"		31	61
Iberis amara (Europe)	h	60	98
"		89	131
Ionospidium acaule (Portugal)	u	61	61
Lobularia maritima (Mediterranean)	r	137	60
"		62	62
Malcomia maritima (south Europe)	h	91	113
"		69	90

TABLE I—*Continued*

SPECIES AND LOCATION	BLOOM- ING RE- SPONSE	EXPERI- MENTAL PLANTS (DAYS)	CONTROL PLANTS (DAYS)
BRASSICACEAE— <i>Continued</i>			
Mathiola bicornis	h	104	118
Raphanus sativus var. French Breakfast (Mediterranean)	h	120	*
“ “ var. White Icicle (Europe)		90	139
CAPPARIDACEAE			
Cleome spinosa (tropical America)	r	125	95
RESEDACEAE			
Reseda odorata (north Africa)	h	124	134
“ “		106	147
CRASSULACEAE			
Sedum coeruleum (Mediterranean)	h	132	182
MIMOSACEAE			
Mimosa pudica (Tropics)	u	197	200
CAESALPINACEAE			
Cassia chamaecrista (central United States)	h	82	103
FABACEAE			
Dolichos lablab (tropical Asia)	u	102	102
Lathyrus odoratus var. (Sicily) var. “Dwarf Cupid”	h	72	92
Lupinus perennis (southern U.S.)	u	121	121
Vicia faba (North Africa and southwest Asia)	u	71	75
TROPAEOLACEAE			
Tropaeolum majus (Peru)	u	60	60
“ “		73	98
“ “		61	75
EUPHORBIACEAE			
Euphorbia marginata (central United States)	r	184	155
“ “		215	139
LIMNANTHACEAE			
Limnanthes douglasii (California to Oregon)	h	60	114
SAPINDACEAE			
Cardiospermum halicacabum (Tropics)	u	127	127
BALSAMINACEAE			
Impatiens balsamina (India, Malaya)	u	88	88
MALVACEAE			
Abutilon hybridum (Subtropics)	u	198	198
Lavatera trimestris (Mediterranean)	h	95	156
“ “		103	129
“ “		98	195
“ “		103	185
VIOLACEAE			
Viola cornuta (Spain)	h	145	*
Viola tricolor (Europe)	h	82	210
“ “		95	171
LOASACEAE			
Mentzelia lindleyi (California)	h	63	109
ONAGRACEAE			
Clarkia elegans (California)	h	109	127
Epilobium adenocaulon (New Brunswick to Colorado)	h	150	*
Godetia amoena (California to British Columbia)	u	71	85
“ “		130	135

TABLE I—Continued

SPECIES AND LOCATION	BLOOM- ING RE- SPONSE	EXPERI- MENTAL PLANTS (DAYS)	CONTROL PLANTS (DAYS)
APIACEAE			
Foeniculum vulgare (Europe)	h	102	122
Trachymene coerulea (Australia)	u	107	117
PRIMULACEAE			
Anagallis linifolia (Mediterranean)	h	108	207
PLUMBAGINACEAE			
Limonium sinuatum (Mediterranean)	u	100	100
APOCYNACEAE			
Vinca rosea (cosmopolitan in Tropics)	u	195	195
CONVOLVULACEAE			
Convolvulus tricolor (South Europe)	h	83	158
" "		67	101
" "		81	107
Ipomoea purpurea (tropical America)	r	300	61
Quamoclit coccinea (Tropics; Arizona, New Mexico)	u	135	64
" "		79	85
POLEMONIACEAE			
Collomia biflora (South America)	h	90	120
Gilia caespitosa (United States)	h	77	*
Phlox drummondii (Texas)	h	90	95
" "		77	125
HYDROPHYLLACEAE			
Nemophila menziesii (California, Oregon)	h	120	128
" "		52	102
Phacelia viscida (Southern California)	h	92	150
Phacelia whittlavia (Southern California)	h	63	125
BORAGINACEAE			
Echium plantagineum (south Europe)	h	66	112
Myosotis scorpioides (Eurasia)	h	43	95
VERBENACEAE			
Verbena hybrida (Argentina)	r	300	195
MENTHACEAE			
Salvia sp., var. Blue Beard	u	128	128
SOLANACEAE			
Browallia speciosa (Colombia)	u	174	174
Capsicum frutescens (tropical America)	h	105	125
Physalis pubescens (North America to Tropics)	u	115	110
Schizanthus wisetonensis (Chile)	h	66	113
" "		112	119
Solanum rostratum (North Dakota to Mexico)	r	157	106
SCROPHULARIACEAE			
Linaria maroccana (North Africa)	h	84	109
Nemesia versicolor (South Africa)	h	90	131
Torenia fournieri (Indo-China)	h	164	184
" "		147	147
ACANTHACEAE			
Thunbergia alata (tropical Africa)	u	83	83
RUBIACEAE			
Asperula orientalis (Eurasia)	h	50	132

TABLE I—*Continued*

SPECIES AND LOCATION	BLOOM- ING RE- SPONSE	EXPERI- MENTAL PLANTS (DAYS)	CONTROL PLANTS (DAYS)
DIPSACACEAE			
Scabiosa atropurpurea (Eurasia, North Africa)	h	95	*
CUCURBITACEAE			
Citrullus vulgaris (tropical Africa)	u	82	82
Cucurbita pepo (Tropics)	u	54	54
Cucumis sativus (south Asia)	u	70	62
CICHORIACEAE			
Lactuca sativa var. longifolia (temperate Europe)	h	159	193
AMBROSIAEAE			
Xanthium commune (east and central United States)	r	152	53
CARDUACEAE			
Ageratum conyzoides (tropical America)	u	98	108
Brachycome iberidifolia (Australia)	h	139	153
Calendula officinalis (south Europe)	u	76	61
" "		79	79
Callistephus chinensis (China, Japan)	h	158	163
" "		150	170
" "		110	120
" "		146	154
" "		164	*
" "		144	182
Centaurea cyanus (southeastern Europe)	h	83	68
Chrysanthemum carinatum (North Africa)	h	99	135
" "		75	140
Coreopsis tinctoria (Ontario to central United States)	h	139	215
Cosmos bipinnatus (Mexico)	r	175	113
Dimorphotheca aurantiaca (South Africa)	h	84	129
Emilia flammea (New World Tropics)	h	99	110
" "		147	147
Gaillardia pulchella (Arkansas, Louisiana, Arizona)	h	159	218
" "		104	165
Helipterum manglesii (Australia)	h	83	105
" "		67	81
" "		66	76
Tagetes erecta (Mexico)	h	72	98
Tagetes patula (Mexico)	r	76	54
Zinnia elegans (Mexico)	u	*	83
" "		*	133
" "		85	85
" "		74	74

### Discussion

It was hoped to discover in these studies an explanation for the different responses of the plants (table I), perhaps in the latitude of native habitat and consequent accustomed day lengths, or in their usual time of blooming whether in the long days of early summer

or in the shorter days of August and September. It might be that certain families would be found to show fixed reactions of a definite sort. But it must be confessed that no sweeping generalizations can thus far be drawn, and the results of experiments are presented now rather as interesting information. Of the 100 species with which this study deals, blooming was accelerated in 41, retarded in 12, not greatly affected in 27. Attention may, for one reason or another, be called to certain families and species. Further studies with these and with others not now mentioned may be expected to bring out other matters of interest. All of the eight species of the Caryophyllaceae thus far worked with have bloomed early when days are lengthened. A few perennial plants of various families were made to bloom in the first season by the use of supplemental light: *Lychnis viscaria*, *Silene vulgaris*, *Epilobium adenocaulon*, *Gilia caespitosa*. Among the plants which were not affected in their blooming or which were actually retarded by increased day length there is a rather large proportion of tropical species; while very few, perhaps not any, tropical species are much hastened in blooming by increased length of light exposure. A few of the species which are distinctly retarded in blooming by the lengthened day deserve mention: *Mesembryanthemum lineare*, *Euphorbia marginata*, *Xanthium commune*, *Cosmos bipinnatus*, *Tagetes patula*. In *Euphorbia marginata* the development of the characteristic white-edged leaves is greatly delayed.

Considerable preliminary experimentation has been carried on which is not recorded in the present paper. Numerous monocotyledons have been worked with, but most of them show no effects of supplementary light. Work has also been done with the common house plants and with most plants of vegetable gardens, but up to the present these species have furnished little of interest.

The studies here recorded are similar to those of the Purdue<sup>2</sup> and Ohio<sup>3</sup> experimenters whose papers were not received in time to be

<sup>2</sup> GREENE, LAURENZ, WITHROW, R. B., and RICHMAN, M. W., The response of greenhouse crops to electric light supplementing daylight. Bull. 366. Purdue Univ. Agric. Exp. Sta. pp. 1-20. 1932.

<sup>3</sup> LAURIE, ALEX, and POESCH, G. H., Photoperiodism: the value of supplementary illumination and reduction of light on flowering plants in the greenhouse. Bull. 512. Ohio Agric. Exp. Sta. pp. 1-42. 1932.

included in the bibliography previously mentioned. Certain of the plants used are the same in all three studies, and results are in general accord. The present writer, however, did not find a single species in which flowers were increased in size or number by the use of supplemental light. It seems highly unlikely on general biological grounds that such a result should be obtained, and it is probable that reports of such increase are based on too few cases to be of consequence.

The study discloses a number of species which do so well in the greenhouse when given supplemental light that they may be used for botanical class work in flower structure. Most noticeable are: *Triticum aestivum* (Marquis and other spring varieties), *Lychnis viscaria*, *Brassica alba*, *Malcomia maritima*, *Lavatera trimestris*, *Nemophila menziesii*, *Phacelia whittavii*, *Callistephus chinensis*, and *Chrysanthemum carinatum*.

### Summary

1. The effect of additional day length produced by artificial illumination during the winter months upon 100 species of greenhouse-grown (chiefly annual) plants is reported.
2. In practically all cases the experimental plants were taller, somewhat paler, less sturdy, and had a poorer root system than the controls.
3. The time from planting to blooming was shortened in 41 species, not greatly affected in 27 species, and lengthened in 12 species.
4. Of the plants hastened in blooming by supplemental light, most are natives of the temperate zone while those which showed no response were largely tropical. A few perennials were brought into blossom during the first season.

UNIVERSITY OF COLORADO  
BOULDER, COLORADO

PERSISTENCE OF SUBSPECIFIC TYPES  
OF XANTHIUM UNDER FIELD CONDITIONS\*

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 456

CHARLES A. SHULL

(WITH THREE FIGURES)

Although the genus *Xanthium* has been monographed twice in recent years (the North American species by MILLSPAUGH and SHERFF in 1919, and all known species of the world by WIDDER in 1923), it would be hard to find any group of plants in a worse state of taxonomic confusion. This statement is not meant as a criticism of the monographers working in this difficult field.

The number of species is not large. MILLSPAUGH and SHERFF (1) recognize twenty-one species in North America, while WIDDER (4) lists twenty-five for the entire world. He added MILLSPAUGH and SHERFF's *X. curvescens* in an appendix, bringing the total to twenty-six species. But the two monographs have only seven species names in common; and two of these, *X. oviforme* and *X. strumarium* of MILLSPAUGH and SHERFF, are considered to be *X. saccharatum* and *X. indicum* by WIDDER instead of identical with his own *X. oviforme* and *X. strumarium*. Moreover, WIDDER's *X. saccharatum* is considered synonymous with *X. pennsylvanicum* by MILLSPAUGH and SHERFF; and in turn, *X. pennsylvanicum* is considered a form of *X. pungens* by WIDDER. Six of MILLSPAUGH and SHERFF's species are placed in *X. saccharatum* by WIDDER, and five others in *X. pungens*. No less than sixteen of the twenty-one species names used by MILLSPAUGH and SHERFF are considered but synonyms for other species by WIDDER.

It is only fair to say that the authors of these monographs had no opportunity to consult with one another during the progress of their work; nevertheless, one wonders how it is possible for such diversity of opinion to result from an examination of the same material. Under the circumstances one cannot speak with great certainty

\* Read before the Illinois Academy of Science, East St. Louis, May, 1933.



about any particular species. There is much variability in the individual species apparently, and some natural crossing. The limits of variability need to be determined under severe tests, and the final revision of the genus may need to be based on a complete collection of all *Xanthium* types into some botanic garden where the habits of growth, responses to variable conditions, limits of variability, and hereditary characteristics for each form can be accurately observed.

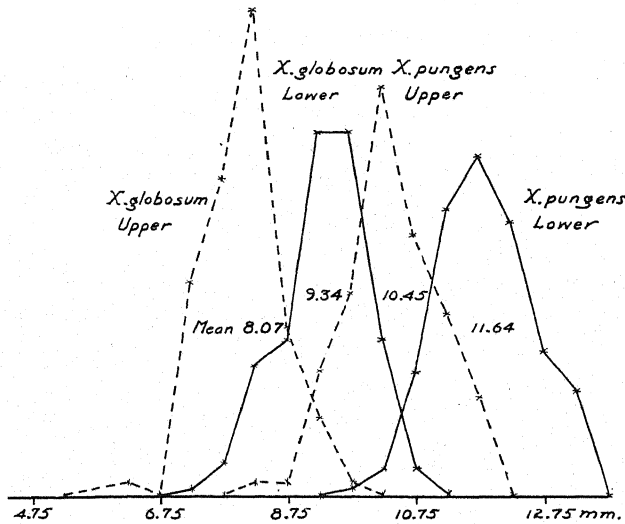


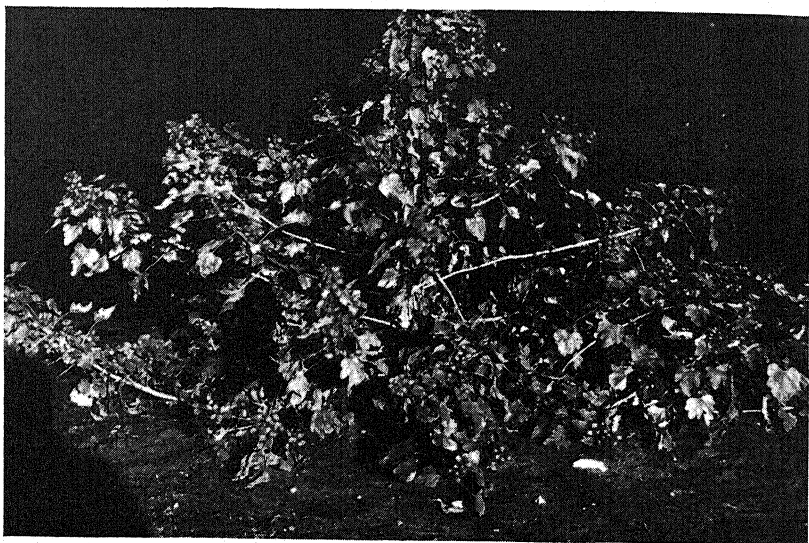
FIG. 1.—Curves of variability in length (in mm.) of upper and lower seeds of *X. globosum* Shull and *X. pungens* Wallr.

Several types of *Xanthium* have been observed in garden and field during recent years. One of these forms it was my good fortune to discover and name. *X. globosum* was first found near Lawrence, Kansas. It was grown for a number of years, and remained true to type generation after generation. MILLSPAUGH and SHERFF have given this form specific rank, while WIDDER, after examining it, calls it a variety of *X. pungens*, *X. pungens globosum*. A statistical study of the length, breadth, and weight of the seeds of *X. globosum* was made, in comparison with what WIDDER considers true *X. pungens*. In figure 1 is shown the curve of variability in length of the upper and lower seeds of *X. globosum* and *X. pungens*, based on the measurement of 200 seeds of each. There is almost no overlapping of

the curves. The curves of variability in breadth and weight show equally well the separation of these two forms. *X. globosum* has very dark colored, sleek, shiny seed coats, while the seed coats of WIDDER'S *X. pungens* are dull grayish brown in color. In view of the fact that *X. globosum* differed in blooming time from *X. pungens*; differed in shape, color, and armature of bur; in color of seed, shape, length, breadth, and weight of seeds, it might well be given specific rank. It is able to maintain itself indefinitely under field conditions. Since it is controlled as to blooming time largely by photoperiodic responses, it tends to maintain its identity in the field, without excluding the possibility of crossing by wind pollination.

Another peculiar type of *Xanthium* which has been under observation for a number of years is the multiple-seeded form originally named *X. canadense globuliforme* by CREVECOEUR, but listed by MILLSPAUGH and SHERFF (2) as *X. chinense globuliforme*. It occurs sporadically in nature and may possibly be a hybrid; but if it is a hybrid it is of a non-splitting type. It reproduces itself year after year in the field, true to type, without any reversion and without any protection of any kind. Each bur usually contains five or six viable seeds. It is a vigorous grower, is enormously prolific (fig. 2), usually fasciated (or very much branched in lieu of fasciation), and is entirely unlike any other cocklebur in nature as to bur construction. While there are always a number of sterile blossoms in each bur, there are usually half a dozen fertile flowers, and no tendency has been noted toward a reduction of this number. If this type were permitted to escape, it could become a most serious pest. It is sturdy enough to maintain itself in severe competition with other weeds, and with other varieties of cockleburs. It will outgrow and outyield any other species of *Xanthium* found in the United States.

A third form which is considered of subspecific rank is a laciniate leaved form which has been described by SHERFF (3) as *X. pennsylvanicum laciniatum*. Similar laciniate forms are described by WIDDER as *X. orientale laciniatum*, *X. riparium laciniatum*, and *X. spinosum laciniatum*. This American laciniate leaved type was first found growing near Onaga, Kansas, by the late Mr. CREVECOEUR, and sent to me some years ago. It has been grown for four generations and has come fairly true to type. It is making itself at home in



FIGS. 2, 3.—Fig. 2 (upper), specimen volunteer plant of *X. canadense globuliforme* Crevecoeur, season of 1932. Fig. 3 (lower), specimen volunteer plant of *X. pennsylvanicum laciniatum* Sherff and Shull, season of 1932.

the field. Its offspring come up each year, and repeat the laciniate form of leaf (fig. 3). A test is in progress<sup>2</sup> at the present time to determine whether 100 per cent of the offspring are true to type, or whether a definite proportion of them revert to the ordinary field type of *X. pennsylvanicum*. Left to itself, there seems very little doubt of its ability to take and hold its place among other weeds, including those of its own genus, as a definite and permanent field type of *Xanthium*.

Such forms raise the whole issue of species concepts. What is the criterion or criteria of species? Should these true breeding forms be considered as mere varieties, or do they possess the necessary distinction and permanence to demand ranking as species? Taxonomists must ultimately settle this question. But unless there are some better standards than immature type and cotype materials stowed away in museums, we shall always have trouble in making a satisfactory classification. It is taxonomic heresy to throw away past work and begin all over again. But in this case, a collection of all species into a single garden might make possible a better definition of species. Ultimately an international congress might have to settle the status of the various true-breeding forms that are now considered of subspecific rank.

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<sup>2</sup> This test involved about 360 plants, the offspring of a single parent plant; 100 per cent of the progeny were laciniate leaved.

CALLIXYLON WHITEANUM SP. NOV., FROM THE  
WOODFORD CHERT OF OKLAHOMA

CHESTER A. ARNOLD

(WITH THREE FIGURES)

The material constituting the subject of this account comprises several pieces of silicified *Callixylon* wood which were collected by Dr. L. B. KELLUM in the Arbuckle Mountains during 1931. All are small specimens broken from larger trunks. None of the primary tissues in the central portions of the stems are included, so the description and the comparisons of this material with other types must be based upon secondary wood characters alone.

While the pieces at hand are small, they indicate having been broken from logs of considerable size. Very large logs as much as 5 feet in diameter are reported from the Woodford chert. These show that there must have been forest trees at the time of this deposition, comparable to the very large trees of *Callixylon newberryi*, the remains of which are common in the New Albany shale of Indiana and the Antrim shale of Michigan. It would seem, therefore, that both of these pre-Coal Measures species produced trees as large as those in a present day forest.

Since only the secondary wood is available, many of the anatomical features of *C. whiteanum* are unknown. It may be stated, however, that this wood presents no marked peculiarities or striking anatomical features, and as far as the width of growth rings, pitting, size of tracheids, etc., are concerned, this species shows but little to distinguish it from any of the better known forms, such as *C. zalesskyi* from the Genundewa limestone of New York or *C. newberryi* from the New Albany shale of Indiana. These forms have been previously described in some detail (1, 2).

The problems of recognizing species from secondary wood characters are frequently stressed in anatomical literature and the difficulties as they apply to *Callixylon* have been previously discussed (2). After a detailed examination of several specimens of *C.*

*newberryi*, it became apparent that such features as thickness of the cell walls, number of pits per group, and width of the poorly defined growth rings usually visible in this wood are not clearly diagnostic and should be used to define species only after lengthy deliberation, and then with extreme caution. The regular distribution of the pits in radially aligned groups on the radial walls of the tracheids is a character confined, as far as the writer knows, to this genus. But the number of pits that make up the groups, or whether they are predominantly in one, two, or three rows per tracheid, are not in themselves characters that will clearly define a species. It should not be inferred from this that different species show no differences in these respects. *C. erianum*, for example, has groups with fewer pits on the average than *C. zaleskyi* or *C. newberryi*, but even here the extremes are about as great in one species as in the other. The number of rows of pits per tracheid is even of less significance, because this is definitely correlated with the size of the cells, and is not a specific character.

Vague growth rings appear to be present in all forms of *Callixylon*, and they can be seen in almost any specimen provided the block is large enough to include the very widely spaced ones. But the single fact that the rings in one block of wood may, for example, average 5 mm. in width and in another block 10 mm. has no specific significance where such variable characters are concerned.

The anatomical feature exhibited by *C. whiteanum* that appears to distinguish it is the structure of the rays as seen in tangential section. Most of the rays are entirely uniseriate, a few are partially biseriate, and occasional ones are entirely so. But the proportion of rays showing complete biseriation is small in proportion to the narrow ones. In *C. newberryi* fully one-half of the rays are completely biseriate. The resemblance to *C. zaleskyi* is much closer, and were the preservation of the latter such that this species could be studied in more detail, it is possible that the apparent differences between it and *C. whiteanum* could be interpreted as within the limits of variation of a single species.

It seems unadvisable to assign the Oklahoma material to *C. zaleskyi*, however, since it shows some features which appear to distinguish it. Furthermore, it comes from a horizon which is probably

distinct from the Genundewa limestone, and it is considered well to adhere to the usual practice of assigning new names to fossil plants of doubtful specific relationships and from distinct horizons. This is done, however, with the understanding that such nomenclature is tentative and subject to such modification as additional information upon the subject might require.

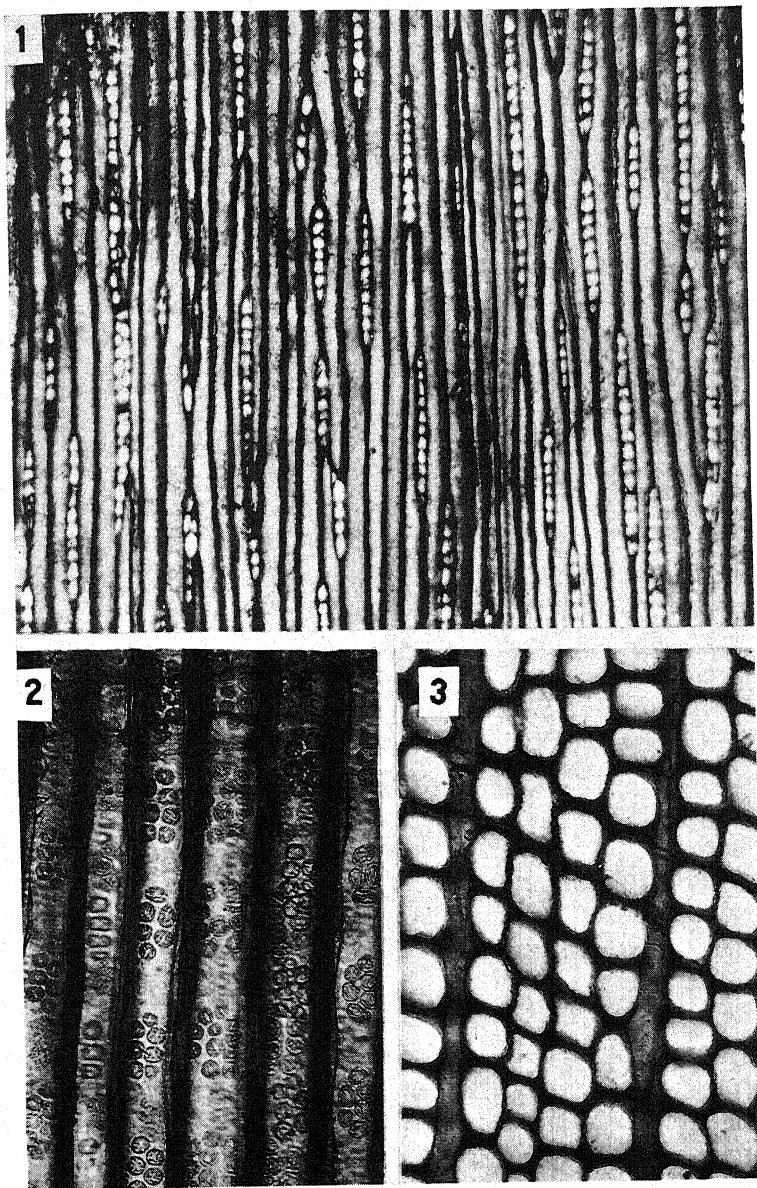
In *C. whiteanum* the rays range from a few (three or four) to seventy or eighty cells in height. The extremely high ones are rare and the majority of them are between ten and twenty-five cells. Such variations as this make concrete statement for the purpose of specific diagnosis rather difficult.

Ray tracheids have not been observed with certainty in *C. whiteanum*, but, judging from their occurrence in other species of the genus, it is altogether probable that they occur in all forms. Their sporadic occurrence in *C. newberryi* was unobserved until after a prolonged examination of exceptionally well preserved material. If such structures are present in the Oklahoma species they are inconspicuous in it also.

In naming this species, it seems appropriate to associate it with the name of Dr. DAVID WHITE, Principal Geologist of the United States Geological Survey.

***Callixylon whiteanum* sp. nov. (figs. 1-3)**

DIAGNOSIS.—Arborescent; trunks large, several feet in diameter. Secondary wood gymnospermous, consisting of tracheids and rays; growth rings present but not strongly developed, irregularly spaced. Tracheids medium sized, varying from 30 to 70  $\mu$  in diameter, square or slightly elongated radially; thickness of wall between lumina of adjacent cells 6 to 11  $\mu$ . Bordered pits in one, two, or three (rarely four) rows on the radial walls of the tracheids; round or hexagonal or slightly appressed vertically if in a wall containing a single row; in radially aligned groups of few to many, but usually 5 to 15. Ray cells 4 to 20  $\mu$  wide, slightly higher; ray tracheids probably present but not clearly determinable. Rays mostly uniseriate but frequently biseriate in part or rarely biseriate for the entire height; few to many (75 or more) cells high. Extremely high rays rare.



FIGS. 1-3.—*C. whiteanum* sp. nov. Fig. 1, tangential section showing predominance of uniseriate rays,  $\times 60$ ; fig. 2, radial section showing disposition of pits in aligned groups,  $\times 150$ ; fig. 3, transverse section,  $\times 125$ .



HORIZON AND LOCALITY.—Woodford chert (probably lower Mississippian), Arbuckle Mountains, Oklahoma (type, no. 15454, Museum of Paleontology, University of Michigan).

In comparing *C. whiteanum* with other known species of the genus, its closest resemblance as shown by the structure of the rays seems to be with *C. zalesskyi* from the Genundewa limestone of New York (1). This comparison is of necessity based upon secondary wood characters alone, and is subject to modification pending the discovery and description of the primary wood of the Oklahoma material. With the exception of the ray tracheids, which are abundant in *C. zalesskyi* but rare or absent in *C. whiteanum*, the general morphology of the rays is similar in the two forms. The pitting shows no features which serve as a basis for distinction.

As already explained, the scarcity of broad biseriate rays in *C. whiteanum* is the most pronounced difference between this species and *C. newberryi*. To the casual observer the similarity in preservation between the two species gives a superficial impression of likeness, which is seen not to exist after a careful microscopic examination. Being calcified instead of silicified, *C. zalesskyi* is in a much poorer state of preservation, and the similarity between this species and *C. whiteanum* is likely to escape the uncritical observer. Concerning size of tracheids, extent of the pit groups, etc., there are no appreciable differences between *C. whiteanum*, *C. newberryi*, and *C. zalesskyi*.

Certain resemblances between *C. whiteanum* and the Russian species, *C. trifilievi*, are noted. Examination of a few sections kindly supplied by the Cambridge Botany School shows the rays of the latter species to be predominantly small. It should be noted that in the original description of *C. trifilievi*, ZALESKY (4) emphasized the small rays.

In a recent work on *Archaeopitys eastmanii*, SCOTT (3) calls attention to the fact that *Pitys*, *Callixylon*, and *Archaeopitys* constitute a unified group. It is unfortunate that the reproductive structures of none of these forms are known, for until then it will be impossible to determine the exact relationship of these forms to either the Cordaitales or the Pteridospermae. An intermediate position is suggested for *Callixylon* because of the diversity shown by the rays. Those

forms with wide rays, as *Pitys antiqua* and *C. newberryi*, suggest pteridospermic relationships, while other forms, as *C. trifilievi*, *C. whiteanum*, and *C. zalesskyi*, are more cordaitean.

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## CHEMICAL COMPOSITION OF CERTAIN AQUATIC PLANTS

HORACE J. HARPER AND HARLEY A. DANIEL

In a previous paper<sup>1</sup> it was shown that the nitrogen content of the soil in ponds was an important factor in the development of a vigorous growth of aquatic vegetation, and since plants are an important link in the growth cycle of certain micro- and macro-organisms found in water, a further study of the composition of aquatic vegetation was made.

Composite samples of all plants which make up the major portion of the vegetation in ponds and lakes near Stillwater, Oklahoma, were collected in June, 1933. All of the plants were approaching maturity and should represent an average condition for this area. Analyses for total nitrogen, phosphorus, and calcium were made on the plant tissue after drying at 105° C., and the results are given in table I.

The nitrogen content of these plants was higher than that which is found in weeds and grasses, although a marked variation occurred in the total nitrogen content of the same kinds of plants secured from different locations. This difference was clearly demonstrated in case of *Potamogeton foliosus*, which was low in nitrogen when grown in sandy soil and high in nitrogen when grown in a dark colored soil higher in total organic matter. Similar results were secured with *Typha latifolia*. Data on the average composition of some common weeds and grasses are given in table II.

The average phosphorus content of the aquatic plants also was higher than that of ordinary forage produced from the growth of cultivated plants. This is an important factor since certain types of macro-organisms depend upon green plants for food, and plant material high in nitrogen, phosphorus, and calcium would be favorable from the standpoint of food supply. A considerable variation occurred in the phosphorus content in the same species, which might indicate the possibility of "luxury consumption" of this element by

<sup>1</sup> HARPER, H. J., Studies on the phosphorus content of certain Oklahoma waters and notes on the development of artificial ponds. Proc. Oklahoma Acad. Sci. 11. 1931.

plants growing on fertile soils, since there was no indication that the absence of other plant foods was restricting vegetative growth.

The data on the calcium content were unusual. All samples of *Elodea canadensis* were very high in calcium while all samples of

TABLE I  
NITROGEN, PHOSPHORUS, AND CALCIUM CONTENTS OF AQUATIC  
PLANTS SECURED FROM LAKES AND PONDS NEAR  
STILLWATER, OKLAHOMA

No.	SPECIES	PERCENTAGE		
		NITROGEN	PHOSPHORUS	CALCIUM
1	Eleocharis	1.410	0.134	0.600
2	Elodea canadensis	1.675	0.231	10.800
3	Elodea canadensis	2.430	0.148	8.440
4	Elodea canadensis	1.990	0.196	8.650
5	Jussiaea diffusa	1.620	0.278	1.065
6	Myriophyllum pinnatum	1.840	0.121	5.920
7	Myriophyllum pinnatum	2.278	0.268	2.150
8	Nelumbo lutea	1.860	0.204	2.770
9	Nelumbo lutea	2.320	0.237	2.945
10	Potamogeton americanus	2.040	0.195	2.240
11	Potamogeton americanus	1.427	0.204	1.602
12	Potamogeton foliosus	2.520	0.492	2.800
13	Potamogeton foliosus	1.650	0.236	1.045
14	Potamogeton foliosus	2.125	0.226	2.765
15	Potamogeton foliosus	1.695	0.167	2.960
16	Potamogeton pectinatus	1.977	0.157	2.996
17	Sagittaria cuneata	2.250	0.220	1.045
18	Sagittaria cuneata	1.819	0.245	0.970
19	Typha latifolia	1.431	0.170	0.378
20	Typha latifolia	0.875	0.126	0.420
21	Typha latifolia	2.348	0.137	0.372
22	Typha latifolia	1.983	0.180	0.354
23	Typha latifolia	3.594	0.304	0.785
24	Spirogyra	1.420	0.131	4.625
25	Nodularia spumigena	2.794	0.364	2.100
26	Spirogyra	0.603	0.071	8.950

*Typha latifolia* were very low in this element. Incrustations on the leaves of *Elodea* plants may account in part for the high calcium content. The calcium content of aquatic plants may be an important factor in the clarification of water, since the water in ponds and lakes which contain no springs usually remains in a turbid condition for a

long period of time following periods of excessive rainfall, when no vegetation is present in the water, while the water in ponds and lakes containing vegetation clarifies rather quickly following rains, except during the winter months when biological activity is at a minimum. Further investigations on the effect of the calcium content of plants in relation to the clarification of turbid water is in progress.

Algae are commonly found in lake waters when the temperature of the water is not too high. Sample 25 in table I which was identi-

TABLE II  
AVERAGE CONTENT OF TOTAL NITROGEN, PHOSPHORUS, AND CALCIUM  
IN SOME COMMON WEEDS AND MATURE GRASSES IN OKLAHOMA

No.	SPECIES	No. OF ANALYSES	PERCENTAGE		
			NITROGEN	PHOSPHORUS	CALCIUM
1	Ambrosia artemisiaefolia	2	1.30	0.22	1.94
2	Andropogon furcatus	26	0.51	.08	0.27
3	Andropogon scoparius	43	0.61	.07	0.27
4	Erigeron canadensis	7	1.81	.27	0.98
5	Lactuca scariola	3	1.63	.28	1.82
6	Polygonum pennsylvanicum	1	2.54	.15	1.55
7	Sorghastrum nutans	6	0.83	.08	0.29
8	Syntherisma sanguinalis	5	1.46	0.19	0.36

fied as *Nodularia spumigena* was highest in total nitrogen. This sample was obtained from a pond which collects a considerable portion of its drainage from barn lots, and consequently the water was high in nitrogenous matter. Sample no. 24 was a spirogyra and was taken from a fish hatchery pond which had recently been fertilized with sheep manure containing a large quantity of alfalfa stems, both of which are high in nitrogen content. Sample no. 26 was also a spirogyra and it was lowest in total nitrogen. It was growing in a pool of water surrounded by sandstone rocks which were very low in phosphorus content, and this probably accounts for the small amount of phosphorus in these plants.

All of the algae were very high in calcium, and when algae are killed by the addition of copper sulphate to ponds and lakes, the organic matter begins to decompose, carbon dioxide production is accelerated, and the calcium compounds which are liberated are

available to flocculate clay particles which remain in suspension. When turbid waters do not contain vegetative plants which can easily be killed by the addition of small amounts of copper sulphate, treatment with this chemical does not increase the rate of settling of the colloidal material.

OKLAHOMA A. AND M. COLLEGE  
STILLWATER, OKLAHOMA

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## BRIEFER ARTICLES

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### CHROMOSOMES OF THE SOY BEAN

(WITH ONE FIGURE)

The diploid chromosome number of *Soja hispida* (Mönch) has been reported as 40 by KARPETSCHENKO.<sup>1</sup> Since this species is very closely related to, if not identical with, the common soy bean *Soja max* (Piper), it was considered desirable to check the chromosome number of the latter by using a common variety. Numerous root tips and flower buds of several varieties were collected and passed through the usual killing and fixing processes, and stained with Haidenhain's iron-alum haematoxylin in an attempt to secure a cross-section of the proper stage of division. One was finally found, of a bud of the Illini variety, which carried several cross-sections of the metaphase of the reduction division of microsporogenesis. In figure 1 the haploid number of 20 chromosomes can be clearly distinguished; therefore the diploid number of 40 chromosomes in the Illini variety of *S. max* corresponds with that reported by KARPETSCHENKO for *S. hispida*.—COLLINS VEATCH, *Argo, Illinois*.

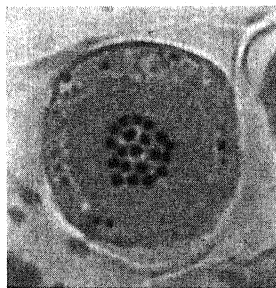


FIG. 1.—Cross-section of metaphase of reduction division of microsporogenesis.  $\times 1600$ .

<sup>1</sup> KARPETSCHENKO, G. D., A report on the chromosomes in *Phaseolus*. Bull. Appl. Bot. and Plant Breed. (Leningrad) 14:143. 1925.

## CURRENT LITERATURE

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### Rusts

In a review of an earlier volume on the rusts by ARTHUR and associates, the reviewer stated that the value of the volume would have been greatly enhanced if a key and list of the known rusts had been included. Fortunately ARTHUR has been able to culminate a long and fruitful lifework with a manual<sup>1</sup> of the rusts in the United States and Canada.

The author aimed to produce a work "serviceable in the determination and collection of rusts by the general botanist," and to "present a classification showing the relation of species and genera as consistent with the present state of knowledge as lineal arrangement permits." In the main, the work abides by the International Rules of Botanical Nomenclature both for parasite and host; however, nomenclatural priority for the rusts is dated from 1753. The author also interprets the concept "perfect state" to be applicable to both uredinia and telia, thus conserving the names under the genus *Uredo* and avoiding substitution of 18 other names in the total of 694 species described. Pycnia and aecia are not recognized in matters of nomenclatural practice. In a few cases, however, the aecial names are used. ARTHUR systematically employs the terminology that he has advocated for designation of the various spore forms of the rusts and of their stromata. The preface includes a brief discussion of the relations of microcyclic (short cycle) and macrocyclic (long cycle) rusts.

An attempt is made to show degrees of relationship for the rusts other than that indicated by hosts. To this end genera are at times divided into sections, recognition of many more separate genera and species therefore being avoided. Species are aggregated "that exhibit correlation, that is, have sufficient points of resemblance to indicate descent from a common ancestor."

Biological and physiological species are not considered. The convention formulated by the American Phytopathological Society in 1925 is adopted as criterion of the species. Genetically, a species is a biological system in space-time characterized by reasonable continuity of interbreeding. So long as this test is not applicable to or has not been applied to the rusts, one convention is as permissible as another in delimitation and definition of species so long as it meets the pragmatic test of convenience and simplicity and is consistently carried out.

The volume is a finished piece of work, and treatment of the subject matter is consistent, precise, informative, and usable. The work represents the fruits of

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<sup>1</sup> ARTHUR, J. C., Manual of the rusts in United States and Canada. pp. xv+438. figs. 487 and map. Purdue Research Foundation, Lafayette, Indiana. 1934.

a life's devotion to the subject, aided by the labors of many others. Its unity is that of one master mind. The value of the manual is enhanced by excellent indices of host and rust names, and by the excellent illustrations by G. B. CUMMINS. Publication was made possible by underwriting by the Purdue Research Foundation.—G. K. K. LINK.

#### Embryologie der Gymnospermen

The work represented by this volume<sup>2</sup> was undertaken originally by DR. STEPHANIE HERZFELD, who had assembled much of the literature and made many drawings, some of them ready for reproduction; but a long illness, ending with her death, came before there was any organization of the literature or writing of the book. Consequently, SCHNARF is responsible for practically the entire work.

The volume is organized under the following headings: male archesporium; formation of microspores; antheridial tapetum; pollen; ovule; female archesporium; macrospores; female gametophyte; female gametes; fertilization; embryo; time of year of various stages. Under nearly all of these headings the material is arranged in the following sequence: Cycadaceae, Ginkgoaceae, Taxaceae, Podocarpaceae, Araucariaceae, Cephalotaxaceae, Pinaceae, Taxodiaceae, Cupressaceae, Ephedraceae, Welwitschiaceae, and Gnetaceae. This organization makes it easy to use as a work of reference, and at the same time indicates the author's view of the rank and sequence of the various components of the gymnosperm phylum. In this feature SCHNARF has followed PILGER's account in the second edition of ENGLER and PRANTL's *Die Natürlichpflanzfamilien*.

While the book is largely a compilation, both in text and illustrations, the organization is excellent and the interpretation, in the opinion of the reviewer, is often more critical and comprehensive than in the original papers. This is doubtless because the author, in making such an extensive survey of the literature, was in a better position to interpret structures and to judge their significance in relation to the whole group.

The survey of the literature is so thorough that it reveals clearly where more work is needed. Consequently, investigators in favorable localities can easily secure material for study, especially since the closing chapter indicates the time of year for various stages in various regions. This feature, not only in this chapter but throughout the book, is so well presented that, knowing the time for one stage, like pollination, the times for other stages can be predicted with more or less probability. With this book for a guide, students in favorable localities can make their investigations very effective.

The book is a notable contribution to our knowledge of the life histories of gymnosperms and its place in the literature of the subject will be permanent.—C. J. CHAMBERLAIN.

<sup>2</sup> SCHNARF, KARL, *Embryologie der Gymnospermen*. Handbuch der Pflanzen-anatomie, K. LINSBAUER. II. Abteilung 2. Teil: Archegoniaten, Band X/2. 8vo. pp. viii+303. figs. 467. Gebrüder Bornträger, Berlin. 1933.



### Systematics of the Ascomycetes

The volume<sup>3</sup> under review is a product of a study begun in 1923 on the systematics of the Ascomycetes. In time the scope of the investigation was limited to a comparative study of Discomycetes, and finally to a study of the supposedly related Discomycetes and Disco-lichenes. The author concludes that the assumption of an extensive relationship between these two groups is erroneous. He proposes to designate the Disco-lichenes and the related Discomycetes as Lecanorales and to drop the term Disco-lichenes. Three main groups of higher Ascomycetes are recognized, and arranged in ascending series as follows: Plectascales, Ascoloculares, and Ascohymeniales. The latter includes, among others, the Discomycetes as its most primitive representatives and the true Pyrenomycetes. The author recognizes operculate and inoperculate Discomycetes, the latter including Lecanorales and the "non-lichenosi." The Lecanorales include the Ostropales (saprophytes) and the Helotiales (saprophytes and parasites). The Helotiales consist of six families, which include the vast majority of non-licheniate inoperculates. The major portion of the volume is devoted to a detailed taxonomic study of the Ostropales and Helotiales. This work is an important contribution to better systematization of the Ascomycetes and includes important theoretical considerations. The usefulness of the volume is greatly enhanced by figures and plates which are well done. There is a good bibliography and an excellent index.—G. K. K. LINK.

### Myxomycetes

A volume<sup>4</sup> devoted to a descriptive list of the known species of Myxomycetes, with special reference to those occurring in North America, has just appeared. It is in essence a revision of the senior author's *The North American slime moulds*, a standard since its appearance in 1922, enlarged to include the Myxomycetes of the world.

The introduction is devoted to discussion of general morphology, including basic definitions and concepts, and of collection and care of specimens. The material is organized on a taxonomic basis with keys to orders, each order with a family key, and each of these with a generic key. The synonymy of each species is given, adding to the value of the keys, and of the excellent descriptions of each species. The reported habitats are recorded.

The appendix lists names not included in the text, a valuable asset to the volume. The bibliography, while extensive, includes only the more important older works, a fuller list of recent articles on the morphology and physiology of the slime moulds, and lists relating to species distribution. The plates consist of

<sup>3</sup> NANNFELDT, J. A., Studien über die Morphologie und Systematik der Nicht-lichenisierten Inoperculaten Discomyceten. *Nova Acta Regiae Societatis Scientiarum Upsaliensis*. Ser. IV. Vol. VIII, no. 2. pp. 368. figs. 47. pls. 20. Almqvist & Wiksells Boktryckeri, Upsala, Sweden. 1932.

<sup>4</sup> MACBRIDE, T. H., and MARTIN, G. W., *The Myxomycetes*. pp. viii+339. pls. 21. Macmillan Co., New York. 1934.

573 illustrations executed by Miss GLADYS BAKER. They are well done and add to the usefulness of the volume. There is a good index. It is fortunate that the work of the senior author is being ably carried on by the junior author.—G. K. K. LINK.

#### Wound compensation, transplantation, and plant chimaeras

An extensive monograph<sup>5</sup> (no. 29 of the *Monographien aus dem Gesamtgebiet der Physiologie der Pflanzen und der Tiere*) has been prepared by KRENKE of the Timiriazeff Institute, Moscow, with the assistance of N. BUSCH, and O. MORITZ of the University of Kiel as translator and editor of the work. It is based on an earlier work, *Chirurgie der Pflanzen*, but so much enlarged and changed that it constitutes essentially a new work.

It is organized in two sections, the first of which (138 pp.) deals with the classification of mechanical influences which affect plants, especially the natural mechanical changes, such as tearing of tissues during normal growth, growth unions (ascidia, etc.), loss of dead or living organs, natural grafts, autotransplantations, formative fusion of tissues in stems and flower heads, and mechanical movements of structural parts such as the migration of nuclei, shifting of position of leaves, and the mechanical effects of wind.

The remainder of the work, which forms the second section, deals with artificial or chirurgic influences. Here are considered the reactions of cells and tissues to wounding, wound healing, regeneration following wounding, and wound compensations of all kinds. A lengthy consideration of transplantation and grafting experiments is given, and an interesting discussion of the physiological effects of stock and scion upon each other. Among these problems of graft partners and their mutual effects are such matters as the transport of organic substance and mineral nutrients from one to the other, changes in frost resistance, active acidity, influence of stock on offspring of scion, leaf color changes, changes in blooming time, effects of different ages of the grafted parts, and stimulus conduction through graft unions. In an appendix the serological relations of graft partners are considered. There is also a section on the practical value of grafting as a horticultural practice. The chapter on Chimaeras considers several types, such as graft chimaeras, stimulation chimaeras, chimaeras as a result of hybridization, and natural accidental and inheritable chimaeras. The last brief chapter considers the introduction of foreign bodies into plants, acquired immunities, internal therapy and related phenomena. The work brings together a very large body of information, and readers will appreciate the full knowledge which Professor KRENKE brought to his task. The literature citations occupy 34 pages. Several indices, genera and species names, authors, and subject matter, make it easy to find any particular information desired. It is, however, far too expensive.—C. A. SHULL.

<sup>5</sup> KRENKE, N. P., *Wundkompensation Transplantation und Chimären bei Pflanzen*. 8vo. pp. xvi+934. figs. 203. Julius Springer, Berlin. 1933. RM. 88.

**Studies of fossil Cycadophytes and Cycadofilicales based  
on epidermal characters**

FLORIN's studies of the epidermis of living conifers have been followed by a volume of similar studies on Bennettitales and related groups.<sup>6</sup> The author shows how the shape of the stomata as well as of the epidermal cells can be used for taxonomic purposes. He shows that a reclassification of Bennettitales will be needed as soon as the available material of coalified leaves is sufficiently examined. FLORIN considers finely preserved coalified plant organs to be more satisfactory than petrifications showing structure. It would be difficult to accept this statement were it not for the excellent photomicrographs which show his specimens.

A shorter paper by the same author<sup>7</sup> describes the stomata of a number of fossil Bennettitales.

The third paper by FLORIN, dealing with fossil Cycadophytes,<sup>8</sup> uses the cuticles for the description of a new species of Bennettitales. The author shows several photomicrographs of stomata at a magnification of 1000 times. They are very clear and so are photographs of hairs magnified 500 times.

Also in the case of Cycadofilicales, the FLORIN method can be used with great advantage, as he demonstrates in a short paper.<sup>9</sup>—A. C. NOÉ.

**A new yearbook of botany**

A new yearbook of botany,<sup>10</sup> somewhat similar to "Progressus rei Botanicae" but more comprehensive and compact, has made its appearance. Volume I reviews the botanical literature published in 1931; and volume II, that of 1932. Each of VON WETTSTEIN's collaborators is an outstanding investigator in his own field of botany, and the following list of chapter titles of volumes I and II, together with the author of each, gives an idea of the merit and scope of the work. (1) Morphology and evolution of cells, by L. GEITLER; (2) Morphology including anatomy, W. TROLL; (3) Phylogeny and reproduction, L. A. SCHLOSSER; (4) Taxonomy, J. MATTFELD; (5) Paleobotany, M. HIRMER; (6) Taxonomic and genetic plant geography, E. IRMSCHER; (7) Physico-chemical foundations of biologic processes, E. BRÜNNING; (8) Cellular and protoplasmic physiology, K. HÖFLER; (9) Water transport and movement of building substances, B. HU-

<sup>6</sup> FLORIN, R., Studien über die Cycadales des Mesozoikums nebst Erörterungen über die Spaltöffnungsapparate der Bennettitales. Kungl. Svenska Vetenskapsakademiens Handlingar. 3d Ser. 12: 1-134. pls. 16. Stockholm. 1933.

<sup>7</sup> ———, Die Spaltöffnungsapparate der Williamsonia-, Williamsoniella- und Wieldiella-Blüten (Bennettitales). Arkiv Bot. 25A: no. 15. pp. 20. Illustrated. Stockholm. 1933.

<sup>8</sup> ———, Über *Nilssoniopteris glandulosa* n. sp., eine Bennettitacee aus der Juraformation Bornholms. Arkiv Bot. 25A: no. 20. pp. 19. Illustrated.

<sup>9</sup> ———, Zur Kenntnis der paläozoischen Pflanzengattungen *Lesleya* Lesquereux und *Megalopteris* Dawson. Arkiv Bot. 25A: no. 19. pp. 23. Illustrated. Stockholm. 1933.

<sup>10</sup> Fortschritte der Botanik. Edited by FRITZ VON WETTSTEIN. Vol. I. pp. 263. Figs. 16. Julius Springer, Berlin. 1932. Vol. II, 1933.

BER; (10) Metabolism of mineral substances, K. MOTHES; (11) Metabolism of organic substances, A. RIPPEL; (12) Ecologic plant geography, H. WALTER; (13) Growth and movement, H. GUTTENBERG; (14) Inheritance, F. OEHLKERS; (15) Phylogenetic physiology, F. OEHLKERS; (16) Ecology, T. SCHMUCKER.

The critical treatment of material and the coherent and readable form in which it is presented distinguish the Fortschritte among abstracting journals. Its value is further enhanced by the promptness with which each issue covers the botanical literature of the preceding year.—A. C. NOÉ.

#### Phylogeny of conifers

A Danish botanist saw in a mountain forest of central Sumatra a peculiar conifer, *Dacrydium elatum*, whose male flowers resembled the strobili of *Lycopodium*. He preserved a large amount of material of the male and female reproductive organs and examined it upon his return to Denmark. The resulting publication<sup>11</sup> gives the interesting facts. He touches the questions of the relationship of conifers with the pteridophytes and the possibility that the conifers might represent the missing link between angiosperms and pteridophytes. The author tries to prove a connection between Lycopodiales and Cordaitales and between the latter and the Lycopodiales. He points to an apparent similarity between certain species of *Juniperus* and some primitive angiosperms. He constructs the following line of evolution: Psilophyta→Selaginellaceae→Lepidospermae→Cordaitales→Coniferae→(Angiospermae?).—A. C. NOÉ.

#### Textbook of systematic botany

In the six years since the appearance of SWINGLE'S textbook, it has proved most serviceable in many laboratories, and this will guarantee a hearty welcome for a second edition.<sup>12</sup> There is no need to repeat here the favorable comments made in reviewing the earlier edition.<sup>13</sup> The pages of the new edition are enlivened by the addition of a number of illustrations, including the portraits of some of the botanical leaders of past generations.

The changes in the new edition are few and relatively unimportant. A few additions have been made, a few minor errors have been corrected, but the whole text remains essentially the same. This will be a matter of satisfaction to many teachers who have found the book useful.—G. D. FULLER.

#### Usefulness of wood in taxonomic descriptions

A series of articles which appeared recently in Tropical Woods illustrate the way in which the wood characters may be used for the description of plant fam-

<sup>11</sup> HAGERUP, O., Zur Organogenie und Phylogenie der Koniferen-Zapfen. Det. Kgl. Danske Videnskabernes Selskab. Biologiske Meddelelser. X, 7. pp. 82. Illustrated. Copenhagen. 1933.

<sup>12</sup> SWINGLE, D. B., A textbook of systematic botany. 2d ed. pp. xv+270. McGraw-Hill Co., New York. 1934.

<sup>13</sup> BOT. GAZ. 86:115-116. 1928.

ilies, genera, and species. Very welcome is a glossary for the wood anatomist<sup>14</sup> which is comprehensive but terse. Another paper<sup>15</sup> explains in few words the value of wood anatomy in taxonomy. An excellent application of the characters of the wood for the systematic description of a family<sup>16</sup> illustrates a paper in a previous issue.—A. C. NOÉ.

#### Textbook of botany

A third edition of HOLMAN and ROBBINS' well known and widely used textbook of general botany<sup>17</sup> has recently been issued. The organization and plan of presentation remain essentially the same as in the previous editions, but such changes have been made by the authors as are justified by the advances of botanical knowledge since the publication of the second edition. The experience of the authors in the use of the book, together with suggestions from other teachers who have used it, have made it necessary to rewrite portions of every chapter. This has added to the clarity and value of the book as a general text while not increasing the number of pages appreciably. A number of the old illustrations have been redrawn or have been replaced by new ones, and there are about 50 additional figures.—J. M. BEAL.

#### Myrrh

A botanical and philological investigation about the source plant of the myrrh of the ancients<sup>18</sup> has been undertaken by an Egyptologist.

The author has compiled all available botanical information about plants that may have supplied the fragrant resin which was a greatly valued article of luxury in classical and biblical times. The myrrh of the ancients contained a volatile oil which was called *στακτή* (Stakte) by the Greeks. This was prepared from the resinous myrrh and had naturally the same source plant. The Egyptians called the resin cntjw.

The author thinks that the source plant might have been *Commiphora molmal* Engler, and suggests that resin of the living plant should be studied in order to establish beyond doubt the connection between the plant and myrrh. SCHWEINFURTH, who collected *C. molmal*, never saw it secrete the resin.—A. C. NOÉ.

<sup>14</sup> COMMITTEE ON NOMENCLATURE, International Association of Wood Anatomists. Glossary of terms used in describing woods. Tropical Woods, Yale University, School of Forestry. pp. 1-12. No. 36, December, 1933.

<sup>15</sup> RECORD, S. J., Rôle of wood anatomy in taxonomy. Tropical Woods. pp. 1-9. No. 37, March, 1934.

<sup>16</sup> GARRATT, G. A., Systematic anatomy of the woods of the Myristicaceae. Tropical Woods. No. 35, September, 1933.

<sup>17</sup> HOLMAN, R. M., and ROBBINS, W. W., A textbook of general botany for colleges and universities. 3d ed. 8vo. pp. xv+626. figs. 463. John Wiley & Sons, New York. 1934. \$4.00.

<sup>18</sup> STEUER, R. O., Myrrhe und Stakte. Wien, Verlag der Arbeitsgemeinschaft der Ägyptologen und Afrikanisten. pp. 48. 1933. RM 5.00.

# THE BOTANICAL GAZETTE

December 1934

## EXPERIMENTAL DATA FOR A REVISION OF THE NORTH AMERICAN WILD ROSES<sup>1</sup>

EILEEN WHITEHEAD ERLANSON

(WITH TWENTY FIGURES)

### Experimental taxonomy

Within the past ten years the problem of species differentiation in several large plant genera has been attacked by a combination of ecological, genetical, and cytological work, with illuminating results. This method of approaching taxonomic problems was originally called genecology by TURESSON (40), a word to which there are some objections in English. BABCOCK has for many years been an enthusiastic exponent of what he designates as cyto-genetics (2), and has carefully analyzed the various types of heritable intraspecific differences in *Crepis* (4). The work of the late DR. H. M. HALL on transplant experiments has contributed valuable information on the evolution of species; this work was aptly called experimental taxonomy (27).

Naturally annual species gave good cyto-genetical data rapidly, and those on *Crepis*, *Nicotiana*, *Viola*, *Triticum*, *Antirrhinum*, *Oenothera*, and *Papaver* are already exhaustive. Many valuable contributions have been made which consist of careful cyto-genetical studies of a few species or species hybrids within a genus (*Phleum*, *Aquilegia*, *Salix*).

Among attempts to study perennial, fruticose, and woody genera,

<sup>1</sup> Paper from the Department of Botany of the University of Michigan, no. 437, representing work done under a National Research Fellowship in the Biological Sciences.

HURST's ambitious plan to make a thorough analysis of the genus *Rosa* has met with several reverses. Specific data on crosses have not been published, nor have the details of intraspecific variation. VAN ESELTINE and NEBEL have begun to attack the species problem in *Malus*, although they can hope to see only three or four generations of apples in their lifetime (41). Valuable results are to be expected from the work in progress at the laboratory of the Carnegie Institution at Stanford on *Pentstemon*, *Hemizonia*, *Zauschneria*, and *Potentilla* (27).

The roses of the North American continent present a problem which demands a new mode of approach. The method that has gradually been worked out is very similar to that expounded by HALL (27), the successive steps of which are: (1) Examination and analysis of herbarium specimens. (2) Field expeditions to observe and collect the living plants in their habitats. (3) Observation of the same living plants in garden culture that were studied in the field, and preparation of herbarium specimens from them. Further steps that have been added to HALL's procedure are: (4) Cultivation of cultures of seedlings grown from the seeds of individual wild plants. (5) Cytological examination and pollen analysis (recently adopted by HALL's co-workers). (6) Interspecific crossing, between closely related species, and between any two species whose natural ranges overlap and which might be expected to hybridize in nature.

All these procedures have been helpful, although the first was the least so because of the fragmentary nature of most rose specimens in herbaria. Herbarium specimens help to give an idea of the geographical distribution of the main rose types and also to give indications of possible localities for collecting trips in unfamiliar regions. Herbarium specimens of *Rosa* should be collected when the fruit is well developed and should consist of a segment of two-year-old wood, with two or three flowering branches bearing ripe or nearly ripe fruit, and a turion of the season. WOLLEY-DOD (44) has emphasized that "more than one specimen from each bush facilitates diagnosis." Good series of specimens from a single locality are also valuable, although not often to be found. The best series of local rose specimens in this country are those collected by SCHUETTE in eastern Wisconsin, now in the Field Museum; DEAM's specimens

from Indiana in the Deam Herbarium; and HOUSE's New York roses in the State Museum at Albany. There are no good representative collections of Pacific Coast roses, although the region from Alaska to central California is probably richer in species than is any other on this continent. The best generally representative collection is that at the University of Michigan.

### Species in *Rosa*

Species of the Linnean type most certainly exist in the genus *Rosa*. Even a brief study of the genus brings out two important facts:

(1) A great diversity of extent among the species. Some species are delimited without difficulty, breeding true and showing only minor variability; others display an enormous degree of variation. In North America the former nearly all have a circumscribed area of distribution, while the latter extend for thousands of miles and may be called polymorphic species, collective species, or species complexes, because taxonomists have given specific rank to so many of their forms.

(2) The existence of a number of variable characteristics common to several of the large polymorphic species which show parallel series of varieties. This was recorded for *R. acicularis* Lindl. and *R. blanda* Ait. from field studies in northern Michigan (12).

One of the most satisfactory definitions of a Linnaean species is that given by VAVILOV, "a separate morpho-physiological system connected in its genesis with a definite environment and area" (43). This definition appeals to the experimentalist because it recognizes the physiological and geographical elements. It has been endorsed by BABCOCK, whose experimental work in taxonomy has confirmed the existence of "unit-groups" of organisms. These natural groups have been characterized in a broad way by BABCOCK (3) as possessing "relative stability, combined with a definite tendency to vary." This broad concept of the species is also subscribed to by CLAUSEN (7a).

The lack of good distinguishing morphological characteristics among the vast number of rose species, and the ease with which inter-specific hybrids are obtained (even when the parents belong to different sections of the genus or differ greatly in chromosome number), are



indications that the genus has been in a rapid evolutionary phase, probably since the Pleistocene, and that most of the species are genetically not distantly related. Similar mutations appearing in different species of the same genus indicate a high degree of genetical similarity (24). The parallel series of variations of the polymorphic species provide further evidence for the assumption that the species of the Cinnamomeae and Caninae at least are closely allied to each other. This agrees with VAVILOV's law of homologous series in variation, for he states (42) that "genetically nearly related Linneons have consequently similar series of hereditary variation."

If these hypotheses be accepted, they will account for the numerous species, varieties, and forms in *Rosa* and for the lack of agreement among taxonomists as to whether the species exist by the hundreds or by the thousands.

The taxonomist must find some way of describing all the multitude of natural forms, and in *Rosa* the problem of how to treat the units smaller than a species is perplexing. GREGOR (23) pointed out that if a varietal name is appended to every specimen which differs in some minor point from the general description of the group, then "every genotype and every clonal modification of an individual would ultimately deserve varietal or sometimes specific rank." To name all the possible combinations of minor characteristics leads to such a state of taxonomic inflation that the categories below the genus lose all practical value. Nor can the morphological criteria alone be accepted as supplying a completely reliable system of classification (23), for morphologically similar forms may differ markedly physiologically, as has been found in *R. acicularis*, *R. woodsii* Lindl., and *R. arkansana* Porter. Probably the most promising method for dealing with the smaller units is that of distinguishing minor variations by numbers or letters, which makes it possible to express different character combinations clearly and succinctly without degrading scientific nomenclature. This has been advocated by VAVILOV (42), HALL and CLEMENTS (26), CLAUSEN (7), and HALL (25). It was recommended for the varieties of rose species by MATTHEWS (31) and is a method that I also would endorse.

### North American rose species in 1925

There has been a great multiplicity of species described for *Rosa*, especially in Europe, yet it is impossible to identify specimens by the keys of ROUX or WOLLEY-DOD (BOULENGER 5). It is possible to describe an endless number of micromorphs in *Rosa*, but when this is done the taxonomic splitters continue to find forms in the field which do not fit any of their described species.

In America relatively few taxonomists have worked on the genus except to describe and to name specimens which were sent to the eastern centers by travelers from the west. The work of GRAY and WATSON belongs in this category. GREENE described several western species. He was a keen observer in the field and would often pick out isolated bushes which were striking in some particular and give them specific rank. RYDBERG believed that any individual that appeared different in detail should be described as a new species; yet he found that several of GREENE's species were not distinctive enough in herbarium samples.

RYDBERG described a number of new species, almost entirely from herbarium material. He has given careful and painstaking analyses of the American species (34), and has discussed the relationships of some of them elsewhere (33, 35, 36, 37). Because he relied on herbarium material which was of very limited extent, he was often much concerned over characteristics that BOULENGER and I have found to be individual variations.

The work of CRÉPIN as it dealt with the American species was good, for he purposely tried to avoid multiplying species. Unlike RYDBERG, he could give varietal rank to plants varying in minor characteristics. He recognized parallel variation in rose species but frequently confused important and minor differences when diagnosing and differentiating species. He badly misunderstood some American species (see also RYDBERG 33) and was handicapped by the fact that he worked too early to profit by the cytological data which has only more recently become available.

When this study was begun, there was such confusion between merely fluctuating and minor variations and differences of specific diagnostic value that almost any series of roses in the field or her-

barium presented some character combinations which had to be either described as new or left undetermined (13). The chief reason for this was the rigid specific descriptions, according to which a few more or a few less hairs or glands or prickles changed the scientific name of a specimen.

### Work of Boulenger and Hurst

HURST's unique theories, cultural experiments, and studies of *Rosa* have given valuable data on several characteristics. He has pointed out new ones, hitherto overlooked, such as the arching habit and the time taken to ripen fruit after flowering (28, 29). He also drastically limits the number of Linnaean species in the genus, which is a sound policy even though several of his groups are artificial.

BOULENGER's work is based mainly upon a painstaking analysis of CRÉPIN's large collection of roses in Brussels, together with field studies upon important characteristics. HURST, BOULENGER, and I all agree with CRÉPIN that there are stable recognizable species in *Rosa* and that the number of species conveniently recognizable in the genus is relatively small. I also subscribe to BOULENGER's statement that, "dans ce genre si polymorphe, la distinction des espèces ne repose que sur des combinaisons de caractères qu'il est impossible d'exprimer en de courtes diagnoses" (5). What CRÉPIN (8) called "les caractères du portrait scientifique des espèces" must be given in lieu of a description of an ideal type. The analytical key which is appended to this paper often transgresses this rule. Yet it may be a better aid in this form to the inexperienced rhodologist, who should aim at writing a better one for his own local forms.

BOULENGER is an eminent authority in herpetology and ichthyology, and in taking up *Rosa* he hoped to be able to strike a new method of attack. He is disappointed in not having been able to do this; the chief thing he has had to do is to demolish, owing to the unreliable and injudicious splitting which has gone on.

CRÉPIN started work on the roses in 1860 and continued until 1900. In 1895 he announced that his monograph would be out in a year or two. Yet his notes were only on the herbarium sheets and in a chaotic state, two or three species names sometimes being placed on one sheet in the European forms. His determinations were often

full of provisions and he was never willing to commit himself. In 1925 BOULENGER suggested to me that it would be a useful contribution to American botany if someone could evaluate the characters used to distinguish species and even sections in RYDBERG's key (34), and to show that many of them are valueless as criteria. Such criticism, even though destructive, is necessary in order to keep old mistakes from being copied again.

BOULENGER strongly advocated the retention of large groups that have some amount of fixity, and when unusual forms are found, to describe them but without giving a name. When minor forms have been described as species it is sometimes expedient to keep the more outstanding ones as varieties, as he has done for *R. glauca* and *R. bakeri*, and as I have done for *R. brachycarpa* Rydb., even though this division has to be somewhat artificial.

#### Cytology and taxonomy of *Rosa*

The taxonomic difficulties of the genus *Rosa* are proverbial, chiefly owing to the unstable, fluctuating, or generalized nature of the characteristics that can be used for the diagnosis of species. In 1922 TÄCKHOLM'S (39) excellent treatise on the cytology of *Rosa* appeared, and it was hoped that the variation in chromosome number would solve the rhodologist's difficulties. Soon afterward HURST (28) proposed a purely artificial classification of the genus, based on chromosome number and upon the assumption of five primitive diploid species. His cytological assumptions have since proved to be invalid (19, 21). In my experience chromosome number has proved to be no more than another important diagnostic characteristic (14). Cytological studies have helped to clarify the situation, but have not seriously affected the classification of the genus as worked out by CRÉPIN in the last century and by BOULENGER on purely morphological lines more recently (5).

The American species fall into three main groups cytologically: the diploids with  $2n = 14$ , the tetraploids with  $2n = 28$ , and the hexaploids with  $2n = 42$ . The hexaploid species *R. acicularis* also has an octoploid race ( $2n = 56$ ) which differs physiologically but scarcely at all morphologically from the more widespread (in America) hexaploid forms.

Pollen grain size varies directly with chromosome number (18) and provides a useful additional method for diagnosing non-typical variants, even in herbarium material.

### Position of North American roses within the genus

The native North American rose species<sup>2</sup> fall into three distinctive and natural sections: (1) *Synstylae* (1 species), (2) *Minutifoliae* (3 species), and (3) *Cinnamomeae* (all other species). This agrees with CRÉPIN'S (10) arrangement except that his section *Carolinae* has been found not to be separated by any reliable characteristics from the section *Cinnamomeae*. *R. gymnocarpa* Nutt. is also now included in the *Cinnamomeae*.

The species of the section *Cinnamomeae* offer the greatest difficulties, being widespread and highly polymorphic. The four species in other sections,—*R. setigera* Michx. (*Synstylae*), *R. minutifolia* Engelm., *R. mirifica* Greene, and *R. stellata* Wooton (*Minutifoliae*),—are readily distinguishable. This paper deals with the section *Cinnamomeae*, unless otherwise stated.

### Diagnostic characteristics in *Rosa*

It is not impossible to find on the same bush two of some of the species listed by RYDBERG (34). Moreover, four or five of these species may occur among the offspring of a single wild rose, as shown for *R. blanda* (14) and for other species in the appended tables of sib cultures grown at Pasadena. This is not a rare phenomenon but rather usual in the groups of *R. acicularis*, *R. arkansana*, *R. blanda*, *R. californica* S. & C. (fig. 1), *R. carolina* L., *R. durandii* Crépin, *R. pisocarpa* A. Gray, and *R. woodsii*, and would probably be discovered in other species if this method were followed.

These variable minor characteristics which give similar series of parallel variations in many American, Asiatic, and European rose

<sup>2</sup> Introduced species. The following exotic species are well established in North America (34):

\**R. multiflora* Thunb.  
*R. moschata* Mill.  
*R. sempervirens* L.  
*R. indica* L.

\**R. laevigata* Michx.  
\**R. bracteata* Wendl.  
\**R. gallica* L.  
\**R. tomentosa* Sm.  
\**R. rubiginosa* L.

\**R. micrantha* Borrer  
\**R. canina* L.  
\**R. cinnamomea* L.  
\**R. pimpinellifolia* L.

\* Frequent in some districts.

species, therefore, should obviously be recognized as part of the variability of each species, and as useless in distinguishing between species or in determining hybridity.

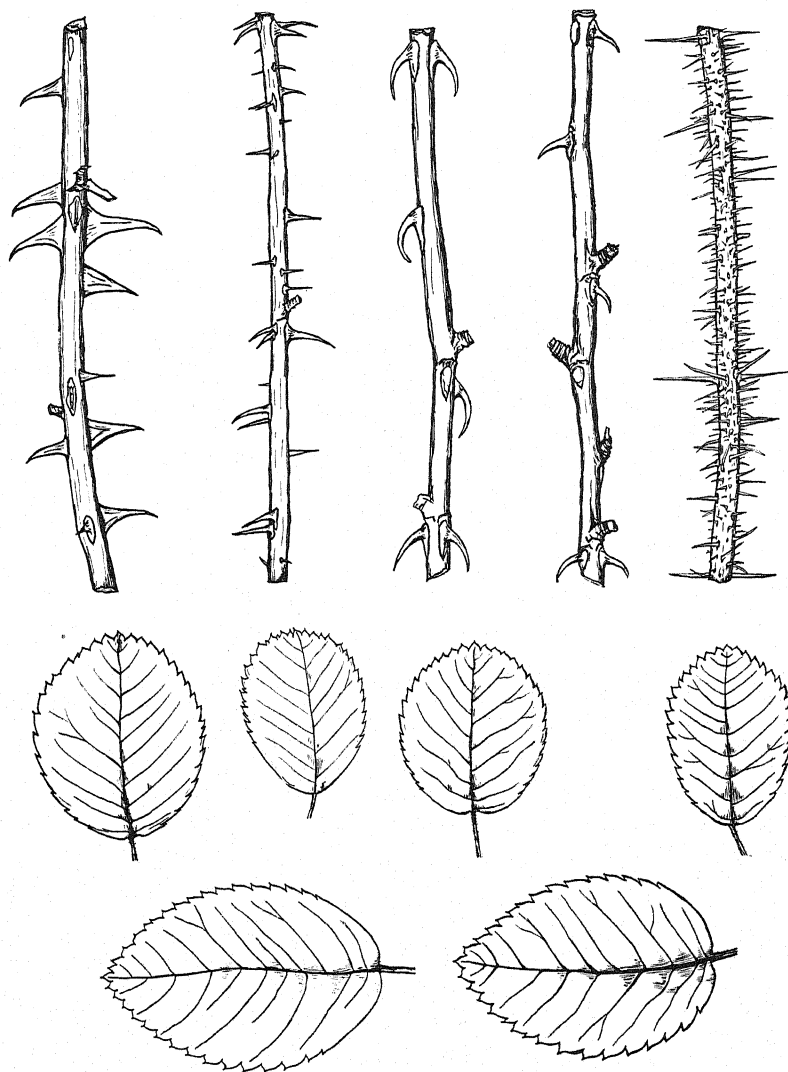


FIG. 1.—Types of stem armature and leaflets from single culture of *R. californica* (no. 12307) grown at Pasadena, Calif. (nat. size.)

## RELIABLE DIAGNOSTIC CHARACTERISTICS

Some good diagnostic characteristics are physiological and therefore are not admissible by the conventional taxonomist. Lack of good characteristics demands their use as an aid in delimiting species:

1. TIME OF FLOWERING.—Among a collection of species under uniform conditions, or in one locality, this is an important and useful diagnostic character (16). Both BOULENGER (5) and I have inde-

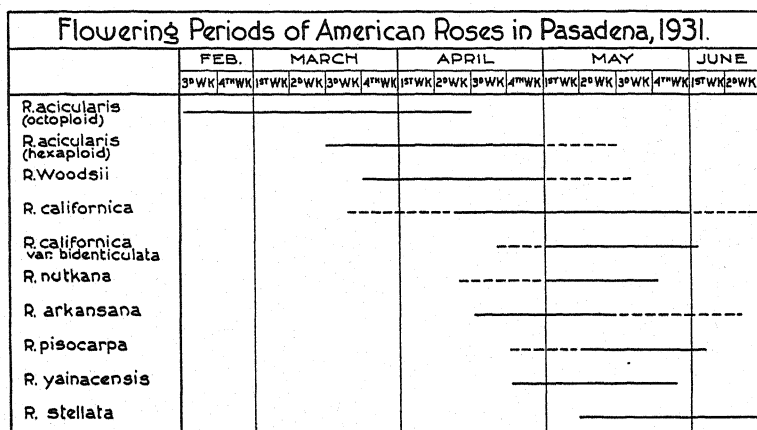


FIG. 2.—Flowering periods of each of 10 native species of *Rosa* at Pasadena in 1931. Main flowering period plotted as unbroken line; stippled lines indicate a few scattered flowers before and after main flowering period.

pendently found that the morphologically simple species flower first and have a more boreal range than have species with more advanced characteristics. The octoploid *R. acicularis* can be distinguished from the hexaploid variety on this account (fig. 2). *R. bidenticulata* Rydb. has also been retained for the reason that it flowers later than *R. californica*.

2. HABITAT.—*R. acicularis*, *R. blanda*, *R. pisocarpa*, and *R. woodsii* belong to littoral habitats (14). *R. arkansana*, *R. californica*, *R. carolina*, *R. durandii*, *R. foliolosa* Nutt., *R. gymnocarpa*, *R. nutkana* Presl., and *R. spithamea* S. Wats. are more usually found in upland or prairie habitats. *R. nitida* Willd., *R. housei* Erlanson, and *R. palustris* Marsh. are found in bogs. This habitat preference distin-

guishes *R. housei* from its western relative *R. arkansana*. When grown at Ann Arbor, one plant of *R. housei* (13207)<sup>3</sup> assumed the normal habit of *R. arkansana* and produced flowering turions with terminal corymbs. It is perhaps an ecophene (40), but more material must be grown to confirm this.

3. HARDINESS AND CLIMATIC TOLERANCE.—Cultures at the Botanical Gardens of the University of Michigan showed that some of the roses of the Pacific Coast region were only partially hardy in southern Michigan. *R. californica*, *R. pisocarpa*, and *R. gymnocarpa* grew very slowly, seldom flowered, and were often cut back by frost or winter killed. *R. woodsii* was very variable in this respect. Plants or seedlings from British Columbia, Washington, the Rocky Mountains, and the Great Plains thrive in Michigan. Plants or seedlings from the arid Great Basin persisted in Michigan but were stunted; they lost their leaves during the summer drought and never flowered. These two physiologically different groups within *R. woodsii* show a parallel series of variations, and cannot be distinguished morphologically.<sup>4</sup>

*R. acicularis* from Alaska ( $2n = 56$ ) responds so rapidly to a rise of temperature in spring that the flower buds are almost always completely frost-killed in April in southern Michigan. The hexaploid requires more or prolonged warmth and always flowers profusely. *R. nutkana* thrives in Michigan, but was stunted and put out little growth in southern California. It also came into leaf later there, in comparison with *R. acicularis* and *R. woodsii*, and flowered later than *R. woodsii*, synchronously with *R. californica* (fig. 2), although in Michigan it flowers earlier than these last two species (16). *R. pisocarpa* was also considerably behind *R. woodsii* in coming into leaf at Pasadena and flowered later than that species.

Seedlings of *R. blanda* from northern Michigan segregated into tender and hardy individuals at Ann Arbor, 300 miles south of their natural habitat. It is interesting to note that the hardy strains of

<sup>3</sup> Culture numbers represent accession numbers at the Botanical Gardens of the University of Michigan.

<sup>4</sup> *R. rubiginosa* and *R. setigera* grow to their normal height, flower profusely, and set much fruit at Ann Arbor, although they are more or less seriously cut back by winter temperatures in some seasons.



*R. woodsii* seem not to have persisted in the region of the Great Basin. Plants from the Mojave desert and from southern New Mexico have not been tested at Ann Arbor. Abortion of hypanthia was frequently observed in the arid regions of the west (15) and may well have been due to lack of water. JOHANSEN (30) found that in *Zauschneria* in California "insufficiency of water underlies the degeneration of the megagametophytes."

The preceding instances all illustrate a feature of what GOOD (22) has called the theory of tolerance; that is, that "morphologically similar species may show wide differences in tolerance." Such characteristics as these, of course, are of little or no aid in the herbarium.

Concerning the morphological characteristics that are valuable I agree with BOULENGER and with HURST:

1. HABIT.—The primitive, strict, straight type of branching is common to most of the American species (fig. 16).

*R. palustris* has zigzag lateral branches.

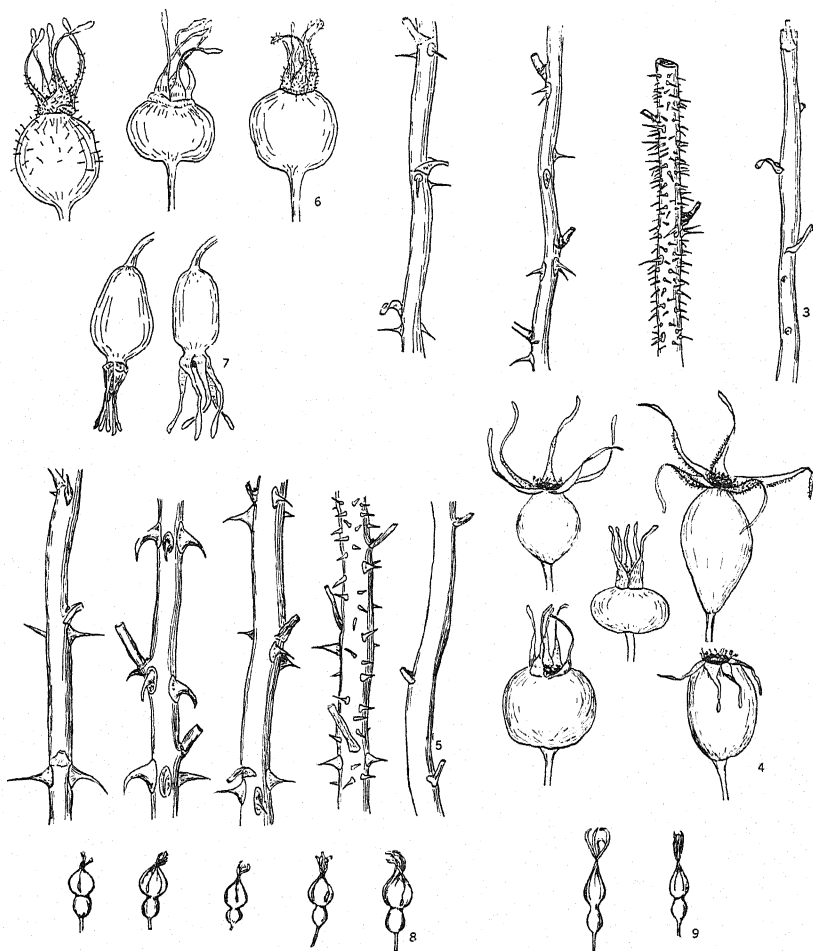
*R. arkansana* and *R. foliolosa* are usually subherbaceous, or suffrutescent, as are some individuals of *R. carolina* and *R. rudiuscula* Greene, with weak decumbent stems.

*R. californica* and *R. pisocarpa* usually have the subcarnuous branches which are stressed by HURST in his EE septet series (figs. 14, 15) although some individuals with the strict habit segregated out in several of my cultures (fig. 17).

2. FOLIAGE.—The *number of leaflets* can be used only in some cases. Leaflets 5-7 on two-year wood and 7-9 or 11 on first-year growth are usual (table I). A few species have been found to have not more than nine leaflets. Ovate leaflets with slightly cordate base are characteristic of *R. pisocarpa* and sometimes of *R. californica* (figs. 1, 10, 11). Lanceolate or elliptic leaflets are usual in *R. foliolosa*, *R. nitida* Willd., and *R. palustris*, but leaflet shape is an extremely variable and unreliable character. All seedlings have glabrous foliage with 3-5 leaflets for the first four or five nodes, and the leaflets are glabrous with glandular-serrulate teeth.

The *number of teeth* per leaflet increases with more highly evolved forms, in BOULENGER'S experience. *R. palustris* alone shows a consistently high number of usually fine, simple teeth, among the roses of the Cinnamomeae (12). *R. pisocarpa* has fine crenate teeth; those

of *R. woodsii* are ascending serrations, usually coarse and acute (fig. 12).



FIGS. 3-9.—Fig. 3, stem armature in *R. blanda*; fig. 4, hip shapes in *R. blanda*; fig. 5, types of stem armature in *R. palustris*; figs. 6, 7, hip shapes in *R. acicularis*; fig. 8, five flower buds of *R. californica*; fig. 9, flower buds of *R. woodsii*. (nat. size.)

*Stipules* are for the most part unimportant for diagnostic purposes. They are characteristically narrow in *R. foliolosa*, *R. palustris*, and *R. californica*, and dilated in *R. blanda* and *R. woodsii*.

3. LENGTH OF FLOWERING LATERALS.—This is correlated with time of flowering and with size of inflorescence. The early flowering *R. acicularis*, *R. nutkana*, and  $\times$  *R. engelmannii* S. Wats. have flowering laterals that are usually 3–10 cm. long. All the other species have some laterals about 7 cm. long and some that may be twice that length. Roses that flower terminally on suckers (flowering turions) may also produce laterals from the base of the main stem which grow to the height of the main plant before flowering.

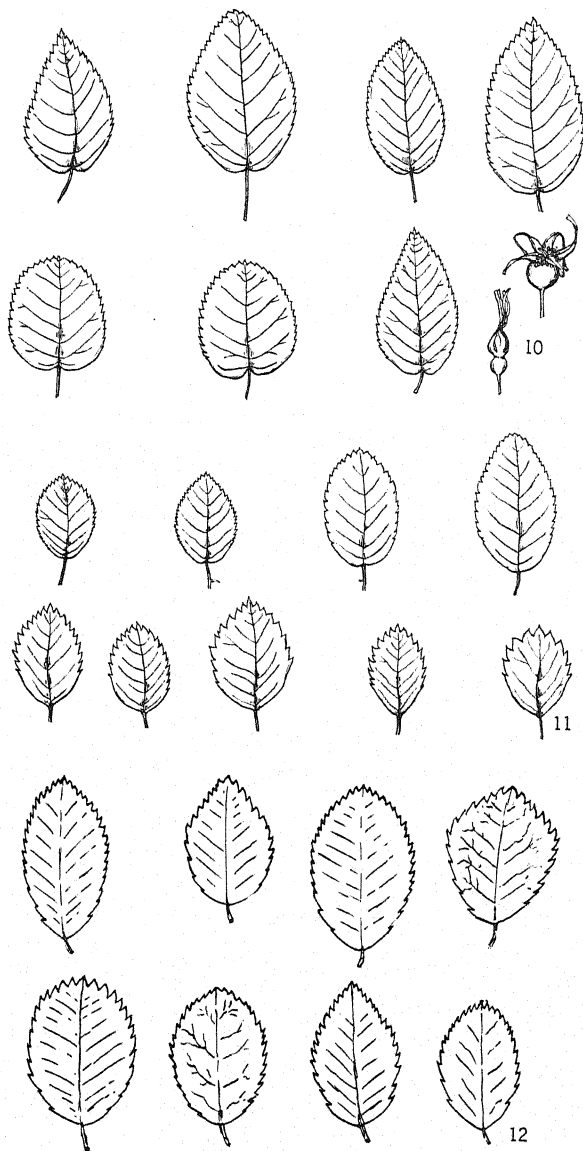
The production of flowering turions after the main flowering period gives what horticulturists call the everblooming habit, and is a valuable diagnostic characteristic for *R. rugosa* Thunb. and for most of the tetraploid American species (except *R. durandii* and *R. yainacensis* Greene).

4. INFLORESCENCE.—Early flowering roses have solitary flowers or 2–3 together on short laterals (for example, *R. acicularis* and *R. nutkana*). The other species of the Cinnamomeae sometimes produce some flowers 1–3 together on short laterals, and others in small or large cymes (4–20 flowers) on longer laterals (table I). A group of flowers subtended by a simple bract, or by a bract with a single terminal leaflet, is considered as a single cyme. Three or four small cymes may cluster at the end of a single lateral, each subtended by a true foliage leaf.

5. LENGTH OF PEDICEL.—This is variable and not of much value, although some species always have stout erect pedicels in fruit. In others the pedicels are erect or pendent in the same culture, even on the same plant.

6. SEALS.—Width of sepals can be used to differentiate between *R. woodsii* and other diploid species. *R. woodsii* has linear-lanceolate sepals 1.5–2.5 mm. wide at the base (fig. 9). No other sepal character has been found to be reliable. In the species of the Cinnamomeae the sepals have almost straight sides; only in *R. californica* (figs. 8, 10) and *R. pisocarpa* is there a tendency for them to be somewhat constricted at the base.

7. PETALS.—The northern and eastern roses and those of the Great Plains have petals 2 cm. or more in length. The Oregon and California species (except *R. nutkana*) have petals usually under 2 cm. long (table I).



FIGS. 10-12.—Fig. 10, leaflets, flower bud, and ripe hip from culture of *R. pisocarpa*; fig. 11, leaflet shapes in one culture of *R. californica* (top row) and *R. californica* var. *bidenticulata* (bottom row); fig. 12, leaflets of *R. woodsii*. (nat. size.)

TABLE I

## NUMERICAL VARIATIONS FROM CULTURE EXPERIMENTS

COLUMNS: 1, NUMBER OF LEAFLETS ON WELL DEVELOPED LEAVES; 2, LENGTH OF TERMINAL LEAFLETS; 3, NUMBER OF TEETH ON EACH SIDE OF TERMINAL LEAFLETS (AVERAGE NUMBER IN PARENTHESES); 4, LENGTH OF FLOWERING LATERALS; 5, NUMBER OF FLOWERS IN AN INFLORESCENCE; 6, LENGTH OF PEDICELS; 7, SEPAL WIDTH; 8, PETAL LENGTH; 9, NUMBER OF STAMENS (AVERAGE NUMBER IN PARENTHESES); 10, DIAMETER OF HIP

1	2 (CM.)	3	4 (CM.)	5	6 (CM.)	7 (MM.)	8 (CM.)	9	10 (MM.)
<i>R. blanda</i>									
5-9	1-5.7	5-20 (12)	3-25	1-20	1-3	2.5-4	2-2.8	85-140 (115)	8-15
<i>R. woodsii</i>									
5-11	0.5-4	5-24 (12)	2-20	1-18	0.3-2	1.5-2.5	1-2.5	35-85 (65)	8-12
<i>R. pisocarpa</i>									
5-9	1-4	8-25 (15)	3-10*	1-9*	0.5-1.5*	2-4	1.2-1.7	44-103 (75)	7-10
<i>R. californica</i>									
3-9	1-4	10-20 (14)	3-30	1-26	0.3-2	3-4.5	1.5-2	65-123 (90)	8-15
<i>R. californica</i> var. <i>bidenticulata</i>									
3-7	0.5-2.5	4-14 (8)	1-10	1-6	0.3-1	3-4	1.2-1.8	65-98 (77)	8-10

\* Probably smaller than normal because of dry atmosphere of southern California.

8. STAMENS.—The number of stamens in each species varies, in some instances within a wide range. Stamen number, however, is a reliable specific character. In *R. virginiana* Mill. the number of stamens is fairly constant and markedly higher than in *R. carolina*.

*R. palustris* has a higher number of stamens than has any other rose of this section. *R. setigera* has an average of 200 stamens (table II).

9. ACHENES.—The number of achenes is roughly 25-50 per cent of the number of stamens. *R. gymnocarpa* has few large ( $5 \times 3$  mm.) usually glabrous achenes. *R. palustris* has numerous small (1.5 mm. long) achenes, which float in water (17).

TABLE II  
NUMBER OF STAMENS IN AMERICAN ROSES

SPECIES AND AVERAGE NO. OF STAMENS	RANGE IN STAMEN NO.																			
	35	45	55	65	75	85	95	105	115	125	135	145	155	165	175	185	195	205	215	220
<i>R. acicularis</i> (octoploid, 75)									—X*											
<i>R. acicularis</i> (hexaploid, 100)									X	—										
<i>R. nutkana</i> (100)									X	—										
<i>R. blanda</i> (115)									X	—										
<i>R. woodsii</i> (65)				X	—															
<i>R. pisocarpa</i> (75)				X	—															
<i>R. gymnocarpa</i> (57)			X	—																
<i>R. yainacensis</i> (70)			X	—																
<i>R. californica</i> (90)					X	—														
<i>R. arkansana</i> (120)								X	—											
<i>R. carolina</i> (105)							X	—												
<i>R. virginiana</i> (140)									X	—										
<i>R. palustris</i> (200)																X	—			
<i>R. setigera</i> (212)																	X	—		
<i>R. minutifolia</i> (45)	X	—																		
<i>R. stellata</i> (175)																X	—			

\* X indicates the average number.

10. DISC AND URCEOLE.—These do not show any significant interspecific differences among the American Cinnamomeae, although BOULENGER (5) found them valuable in distinguishing between some European species.

#### MINOR AND UNRELIABLE PARALLEL VARIATIONS

1. HEIGHT OF STEM.—This is a characteristic that must be treated carefully. It has been found that many of our species segregate dwarfs in culture (fig. 18), so that dwarfness is not a diagnostic char-

acteristic. Some species seldom reach over 1 m. in height, however, whereas under favorable conditions others may attain 2-3 m. or more.

2. ARMATURE.—There are three main types of armature:

(a) Stems normally bristly. A seedling and primitive armature type (5). For example, *R. acicularis*, *R. arkansana*, *R. blanda* (fig. 3).

(b) Stems with bristles and usually terete slender prickles intermixed: heteracanthic. For example, *R. californica*, *R. gymnocarpa*, *R. rugosa*, *R. woodsii*.

(c) Stems with well developed prickles that are usually strong, flattened, and enlarged at the base. These plants fall into two groups: (1) Bristles and stout prickles intermixed throughout: heteracanthic; for example, *R. californica*, *R. durandii*. (2) Bristles usually absent except in the first season and at the base: homoeocanthic. The only true homoeocanthic North American species is *R. setigera*; in the Cinnamomeae, *R. nutkana*, *R. palustris*, *R. pisocarpa*, and *R. virginiana* approach this condition (5, II, figs. 7 and 8 for *R. canina*).

A plant of any armature type may sometimes be completely unarmed; again, only the twigs may be unarmed, or the armature may appear on the lower half of the plant only.

3. FORM AND DIRECTION OF PRICKLES.—The bristles and prickles of any of the armature types may be straight, ascending, reflexed, or hooked (fig. 5, *R. palustris*). Prickles on the same bush frequently vary in form and direction, so that it is useless to distinguish species by this characteristic. Armature is largely a matter of degree.

4. INDUMENT.—The presence or absence of hairs, glandular hairs, or glandular granules on stem, foliage, stipules, hypanthia, pedicels, or sepals is found in varying degrees in all the species. This was reported by SCHNETZ (38) to be an individual peculiarity in roses, but is still used in keys. Simple or compound serrations on the margins of leaflets and gland-tipped serrations often vary on different parts of the same bush, and on different sibs of cultures. Some species are reported not to have glands on the leaflets (except as seedlings); for example, *R. cinnamomea* L., *R. rugosa*. Should a plant of either be found with glandular foliage, however, this would not exclude it from

the species, because this type of covering is a regular part of the parallel variation of the genus. *R. palustris* and *R. virginiana* have not been found with glands on the leaflet surfaces, although an example of each has been observed in herbarium material with finely gland-serrulate leaflets.

5. SHAPE OF RIPE HIPS.—A cursory examination of any series of living roses will show that the hips vary from depressed globose to globose, elliptic, pyriform, and urceolate, and that they often vary on individual bushes and among plants belonging to the same species. In no American species is there a single hip shape. This characteristic is of almost no practical value in distinguishing species (figs. 4, 6, 7). BOULENGER told me that there is not a single rose group in Europe in which a species can be based upon the shape of the fruit (see also BOULENGER 5, I).

6. POSITION OF SEPALS ON RIPE HIP.—As shown in the tables, sepals may be erect, spreading, or reflexed on the full-grown hips in different individuals of the same culture in *R. woodsii*, *R. pisocarpa*, and *R. californica*. This is also true for different hips on the same plant in several groups. The Carolinae were distinguished from the Cinnamomeae largely because their sepals were supposed to be reflexed and deciduous in fruit. In *R. palustris* (section Carolinae of authors) sepals frequently remain erect and do not always fall. Plants of *R. woodsii* and of *R. pisocarpa* (fig. 10) (section Cinnamomeae) have been observed with spreading and deciduous sepals.

7. POSITION OF ACHENES ON WALL OF HYPANTHIUM.—This character was used by CRÉPIN to distinguish further between the sections Carolinae and Cinnamomeae. In *R. palustris* achenes are frequently attached to the side wall of the hypanthium. Plants can be found in *R. blanda*, *R. woodsii*, *R. pisocarpa*, and other species of the Cinnamomeae with achenes at the base of the hypanthium only. BOULENGER (6) also found this to be an inconstant character.

#### Experimental tests of status of some American species

It is difficult to present convincing evidence for the existence of variations on single individuals in prickles direction and form, leaf covering, glandulosity, sepal position, and hip shape, although anyone can quickly convince himself by inspecting living rose bushes.



In this paper an attempt will be made to prove the unsound nature of such characters for distinguishing between species, by tabulating the combinations of those that appeared in cultures of seedlings from single wild female parents. The objection might be offered that these various strains were kept alive by cultivation but would die out in nature. This can hardly be sustained when we remember that these are all minor variations with no apparent survival value (unless dwarfness might be excepted); and that nearly all the variants were observed in nature, sometimes in small areas (12, 15).

A second striking fact, beyond the existence of parallel series of variations in several of the collective species, is the existence of a variable amount of gametic sterility and of chromosome aberrations among some members of almost any species. All these phenomena have been attributed to hybridization between different species in nature, an assumption which needs to be reconsidered in the light of recent data.

#### VARIATION IN PROGENY OF WILD ROSES

*R. blanda* Ait.—The segregations found among the progeny of a wild plant of *R. blanda* var. *glandulosa* Schuette have been reported (14). Since then a more complete analysis of the largest culture (3753/7) has been made (appendix I, table A). It contains plants which would be classified in the field or herbarium as *R. blanda*, *R. subblanda* Rydb., *R. blanda* var. *glandulosa*, *R. blanda* var. *hispida* Farwell, *R. acicularioides* Rydb., and *R. palustriformis* Rydb. These four species and two varieties are distinguished solely by one or more of the minor variable characteristics which have been listed under "unreliable parallel variations."

In order to give, at a glance, the variation discovered in such a culture, these characteristics have been designated by a system of numbers. The numbers have been grouped in columns headed by letters which stand for the part of the plant dealt with (table A). In this way the formula for any individual plant can be written in brief form and can be compared with that of any other. The explanation of the symbols and the formulas for some individual cultures are given in appendix I. Each individual has been determined in most cases by RYDBERG's key (34), and the binomial name thus obtained is given in the last column.

The result of pollen and achene analysis in culture 3753/7 of *R. blanda* showed a great variation in sterility. Figure 13 shows the

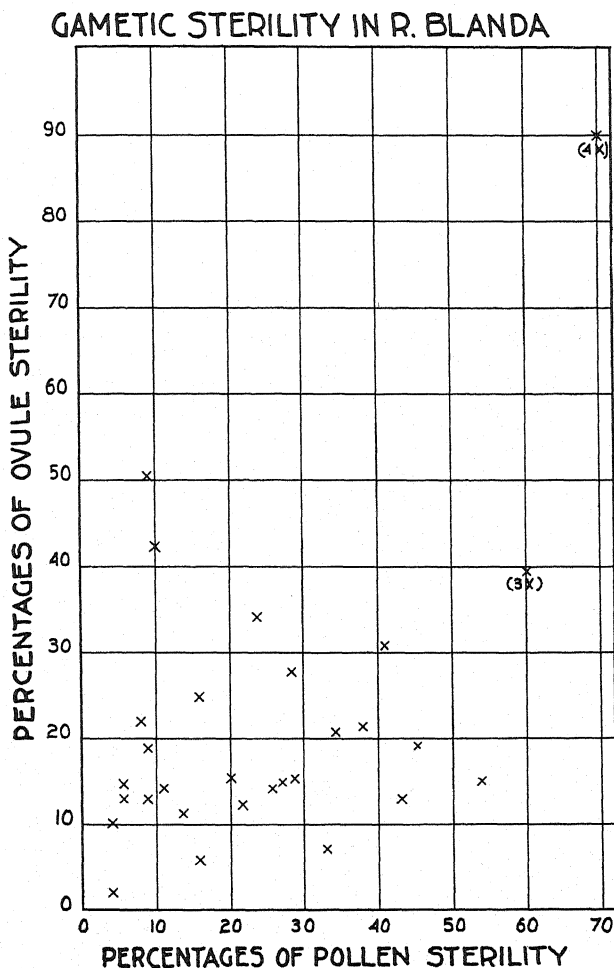


FIG. 13.—Ovule and pollen sterility in 31 plants of *R. blanda*, from culture 3753/7 plotted synchronously for each plant. Data taken in 1930.

male and female sterility percentages for 31 plants, plotted synchronously. No correlation is brought out. Apomixis was found once to occur in the plant shown on the chart with 53% sterile pollen and 15% empty ovules, but has not been obtained again in this plant nor

in any of its sibs. Among 51 plants, 40 had 4-33% of the pollen grains empty, 10 had 34-65% empty pollen, and one plant had 66% bad pollen. Five plants had 50% or more of bad pollen, and of these three were diploid ( $2n=14$ ), one was triploid ( $2n=21$ ), and one was tetraploid ( $2n=28$ ). The semisterile diploid plants showed multivalent groups of chromosomes at diakinesis (19, 21), owing to reciprocal translocations. This is now known to be a widespread phenomenon and is usually not a result of interspecific hybridization. The triploid plant is trivalent in synapsis and apparently arose from the union of a haploid and an unreduced diploid gamete (21). The tetraploid plant flowers first in the culture (it is a dwarf) and seldom sets fruit. This plant probably originated in the union of two unreduced gametes. The female parent of this culture is diploid and has only 15% of bad pollen. This is another example of the rule announced by DARLINGTON (11) that the fertility of a tetraploid is inversely proportional to that of the diploid from which it arises.

The type specimen of *R. blanda* is a small unarmed spray, yet in all parts of its range this rose is frequently bristly and sometimes has weak infrastipular prickles (fig. 3). Diploid *R. blanda* sets a good crop of achenes with pollen from hexaploid *R. acicularis*, and because these two species occur together in the Great Lakes region I at first attributed the great variability of *R. blanda* in northern Michigan to hybridization with *R. acicularis*. Since then a culture of seedlings has been raised from a plant of *R. blanda* from the Gaspé Peninsula, Quebec, where *R. acicularis* is not found. They also show great variability (appendix I, table B); they are nearly all bristly but do not have the elliptical pendent type of hip common in northern Michigan. Several plants in the Canadian culture have bristles on the hypanthium, a characteristic that is one of individual variation in *R. blanda* as in *R. acicularis* (fig. 6), *R. carolina*, *R. nutkana* (13, 15), and other roses (5).

*R. woodsii* Lindley.—In the Great Basin of Utah and Nevada, the common roses at low altitudes all belong to the large diploid group of *R. woodsii*. Species belonging to other groups are not found in Nevada; and in Utah, although hexaploid forms occur, they are limited to high altitudes. In 1928 seeds were collected in these regions (15), and the offspring of several individuals were grown at the experi-

mental garden of the California Institute of Technology in Pasadena, at the invitation of DR. T. H. MORGAN. Most of these seedlings flowered for the first time in 1931 and were then examined. If the variability of *R. blanda* were due to hybridization with other species, then one would expect greater uniformity among the offspring of the western diploids, which are isolated geographically.

The offspring of plants which were identified in the field by RYDBERG's keys as *R. fendleri* Crépin, *R. macounii* Greene, *R. puberulenta* Rydb., *R. pyrifer* Rydb., *R. salicetorum* Rydb., and *R. woodsii*, not only showed as much variation as those of *R. blanda* but had the same variations morphologically. Single cultures from Utah and from Nevada contained some plants with glabrous, some with pubescent, and others with glandular foliage. Among offspring of single plants some were almost unarmed, others were densely bristly with weak paired prickles. Straight and curved prickles, glabrous and glandular sepals, and globose and pyriform hips also appeared in the same cultures (appendix I, tables C-J). In the fragments found in herbaria, each of these variants had been given a separate specific name; consequently, when other combinations were found they had to be described as new species.

Nine offspring of a single plant (culture 12191) from Salt Lake City can be placed in seven different species according to RYDBERG's keys. In four of the six plants that flowered in 1931, more than one-third of the pollen grains were empty. A culture of 21 plants from Nevada (12205) could be classified in five species; among 15 plants that flowered, six had less than one-third of bad pollen (table III), eight had approximately 50% bad, and one had 90% bad and was a triploid from the union of one unreduced diploid and one haploid gamete (21). Giant pollen grains (probably diploid grains) were observed in the pollen of three of the semisterile plants (table IV). Two of the semisterile plants which were examined cytologically showed quadrivalent groups in a few of the microspore mother cells at diakinesis (21). The eleven offspring of another plant (12207, which grew near 12205) could be classified in three species, and the pollen of the four examined showed only 5-12% of empty grains. Culture 12209, also from near Reno, contained 52 plants that could be classified in six species. Among ten plants that flowered in 1931,

seven had less than 33% bad pollen, two had about 50%, and one was a triploid with 88% bad pollen. Similar variations were shown in other cultures from this region (appendix I). A small percentage (0.5-0.6%) of giant grains was observed in pollen of plants with only 20-29% of empty grains.

TABLE III  
VARIATION IN POLLEN STERILITY IN CULTURES OF  
*R. WOODSII* (RANGE 3-95%)

CULTURE	PERCENTAGE		
	0-33	34-65	66-100
I2191.....	2	4	.....
I2205.....	6	8	I *
I2207.....	4†	.....	.....
I2209.....	7	2	I *
I2211.....	.....	2	.....
I2212.....	I	.....	.....
I2218.....	I	I	.....
I2220.....	7	3	.....
I2227.....	I	.....	.....
Totals.....	29	20	2

\* Triploids.

† Range, 5-12%.

TABLE IV  
OCCURRENCE OF GIANT POLLEN GRAINS IN  
CULTURES OF *R. WOODSII*

CULTURE	NUMBER OF PLANTS	DEGREE OF STERILITY	PERCENTAGE OF GIANT GRAINS
I2191.....	2	Semi	0.3-0.6
I2205.....	2	Semi	1.4-2.1
I2220.....	I	20%	0.59
I2221.....	I	20%	0.6
I2227.....	I	25%	0.6

*R. pisocarpa* A. Gray.—This species did not thrive at Pasadena, so that the length of flowering laterals and size of inflorescence were less than would be normal in a moister region. The cultures varied less than those of *R. woodsii* and *R. californica*, but sufficiently to show that absence of armature, hip shape, indument of sepals, and

position of sepals on the fruit are unreliable characteristics on which to base specific differentiation (appendix I, tables K-O). *R. anacantha* Greene, *R. copelandii* Rydb., *R. eastwoodiae* Rydb., *R. pringlei* Rydb., and *R. ultramontana* (S. Wats.) A. Heller are distinguished from *R. pisocarpa* by such characteristics. Among 23 pollen analyses that were made from all cultures, 14 plants had less than 33% bad pollen, six had between 50% and 60%, and three had approximately 70%. These last three were all triploids.

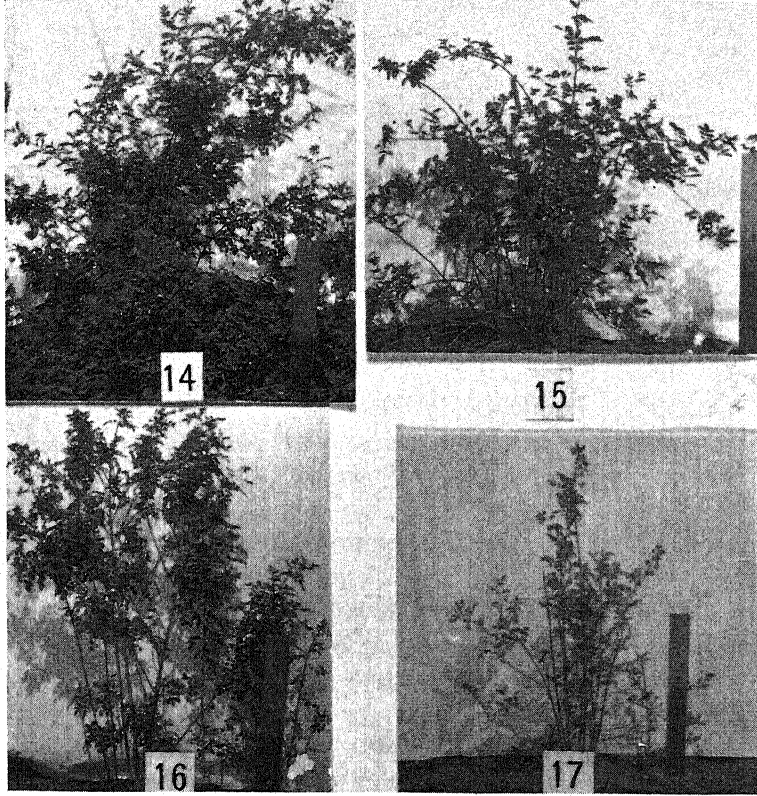
*R. californica* S. & C.—The tetraploid species *R. californica* showed great variation in minor characteristics and gave a series of variants parallel with those found in *R. woodsii*. The stem armature and covering of foliage are more complicated and more intergradations are possible in this group. Some dwarfs appeared in each culture, and a few segregates with strict instead of arched branches (figs. 14 and 17, and appendix I, tables P, Q, R, S).

Culture 12307 contained 44 plants, offspring from a single bush near Upland, San Bernardino County, California. The offspring had a fairly uniform appearance, except for two dwarfs. Yet when details of armature, leaflet, sepal and pedicel covering, and fruit shape were analyzed, as they would be in herbaria, the plants could be classified (with varying degrees of certainty) as belonging to ten of the species given by RYDBERG (34). It is no wonder that California botanists have not taken up these species, but prefer, correctly as I believe, to call them all *R. californica*.

Two small cultures from the San Francisco Bay region showed five plants out of six with about 50% of the pollen shriveled. Thirty plants out of thirty-one that were examined in the culture from Upland had less than 25% of empty grains; one plant had 35%. Variability in the minor characteristics is therefore not necessarily correlated with gametic sterility.

A small culture (12314) from a plant at Hesperia, San Bernardino County, California, consisted of plants about 7 dm. high with slender strict stems (fig. 17). The prickles on all were slender and terete, and leaflets were small, usually about 1.5 cm. long, glandular, and coarsely toothed with usually less than ten serrations each side (fig. 11) (appendix I, table S). These plants were tetraploids and resembled some of the dwarf segregates in cultures of *R. californica*. They were

strikingly distinct in habit from typical *R. californica* and agreed with RYDBERG's description of *R. bidenticulata*. RYDBERG's type was from Shasta County, and it is possible that this depauperate form is an ecotype of *R. californica* adapted to higher altitudes. Until more is



FIGS. 14-17.—Fig. 14, subcernuous habit of *R. californica*; fig. 15, subcernuous habit of *R. pisocarpa*; fig. 16, *R. woodsii*, strict habit; fig. 17, *R. californica* var. *bidenticulata* with strict habit (photographs by DR. G. W. BEADLE, at Pasadena, Calif.).

known about its range and variability, it is advisable to retain it as a variety, *R. californica* var. *bidenticulata* (Rydb.), comb. nov. (*R. bidenticulata* Rydberg, North Amer. Flora 22: 518-519. 1918).

*Other species.*—The preceding examples demonstrate so clearly the fact of similar parallel variations of minor characteristics in different

rose species that details of the analysis of cultures will not be given for other groups.

There are cultures at the Botanical Gardens of the University of Michigan which show the same thing in *R. acicularis*, *R. arkansana*, *R. durandii*, *R. carolina*, *R. palustris*, and *R. virginiana*.



FIGS. 18, 19.—Fig. 18, tall and dwarf plants in single culture of *R. carolina* at Ann Arbor, Michigan; fig. 19, *R. alcea*, dwarf ecotype species related to *R. arkansana*.

In cultures of *R. acicularis* plants appeared that would be classified as *R. acicularis* var. *lacorum* Erlanson, *R. acicularis* var. *rotunda* Erlanson, *R. acicularis* var. *sayiana* Erlanson, *R. bourgeauiana* Crépin, and *R. collaris* Rydberg.

*R. ratonensis* Erlanson 1928 when self-pollinated gave plants answering to the description of *R. arkansana*, *R. conjuncta* Rydberg, *R. ratonensis*, *R. suffulta* Greene, and *R. suffulta* var. *valida* Erlanson.



Two out of four plants obtained from selfing the dwarf *R. relict*a Erlanson could be classified as *R. suffulta*, one as *R. arkansana* (which set no fruit after flowering), and one as *R. rudi*uscula Greene.

Seedlings in a culture of *R. durandii* from Oregon have not yet flowered, but they show great variation as to covering. Some have pubescent stems and prickles, others are glabrous. Some are very prickly and one almost unarmed.

Cultures of *R. carolina* from individual wild plants contained offspring that could be classified (according to RYDBERG) as *R. gemella* Willd., *R. lyoni* Pursh, *R. nanella* Rydb., *R. petiolata* Rydb., *R. serrulata* Raf., and *R. subserrulata* Rydb.

Cultures of *R. palustris* sometimes include specimens that agree with the descriptions of *R. dasystema* Raf., *R. floridana* Rydb., and *R. obtusiuscula* Rydb.

Cultures of *R. virginiana* show less variability. Some plants are low growing; others may have somewhat pubescent foliage (*R. lyoni* (35) or somewhat pyriform fruit (*R. bicknellii* Rydb.).

Field studies have shown that these variations and many others and numerous recombinations of characteristics also occur in nature in the preceding species as well as in *R. gymno*carpa and *R. nutkana* (12, 14, 15).

BOULENGER, working with the extensive herbarium material of CRÉPIN, has found that *R. alpina* L., *R. pimpinellifolia* L., and others also show these same minor morphological variations. His observations have constrained him to reduce numerous so-called species to synonymy.

Culture experiments and field studies indicate that there are relatively few rose species in North America, but that these are highly variable and heterozygotic for the minor characteristics.

### Hybridization

Hybridization has undoubtedly taken place freely between species of the section Cinnamomeae. The whole question of natural interspecific hybridization in *Rosa* is highly speculative (5, II). Intermediate types have usually been judged as hybrids, and the supposed identity of the two parents guessed at (34, 35, 36).

Several interspecific pollinations have been made at the University

of Michigan. Thanks to the cooperation of DR. WILLIAM CROCKER and the staff of the Boyce Thompson Institute for Plant Research, who germinated most of the hybrid achenes, several good cultures of  $F_1$  plants have reached maturity in the past two seasons. These crossings were mostly made in 1929 and 1930. Pollen has been ob-

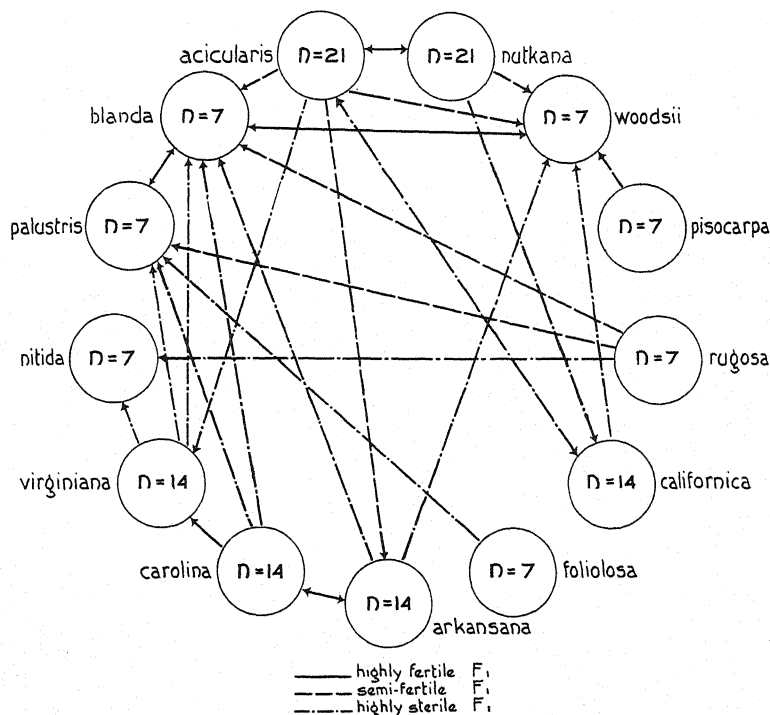


FIG. 20.—Diagram of hybridizations in section *Cinnamomeae*. An unbroken line shows that resulting  $F_1$  has  $1/3$  to  $2/3$  of pollen empty; line of dots and dashes shows that the  $F_1$  has almost all bad pollen; arrows indicate direction in which pollen was carried in making the cross.

tained from several of these interspecific hybrids, which may be conveniently arranged in three classes with regard to pollen fertility: (1) fertile, less than one-third bad pollen; (2) partially sterile, one-third to two-thirds bad pollen; and (3) sterile, over two-thirds bad pollen. These results concerning 13 species are shown diagrammatically in figure 20.

The hybrids will be analyzed in detail as they reach complete ma-

turity. Figure 20 shows that the fertility of hybrids between species having the same chromosome number cannot be predicted. Species possessing the same chromosome number that grow together in nature usually give highly fertile  $F_1$  hybrids, and such hybrids are frequent enough in some regions to be recognizable in the field. BOULENGER (5, II) would eschew the practice of giving binomial names to hybrids, because the diverse forms shown by many of them tend to lead to an ever increasing multiplication of Latin names, a danger that must be avoided. In this paper, however, some of the  $F_1$  hybrids between common American species are designated by binomials already in use, because culture experiments have shown these species and the  $F_1$  hybrids to be almost identical.

#### INTERSPECIFIC $F_1$ HYBRIDS IN NATURE

Since most of the wild roses are highly heterozygotic for minor characteristics and do not breed true, it is not surprising that the  $F_1$  cultures are also far from uniform. Many of them show some uniformity in habit and general appearance, except for an occasional aberrant individual. Minor characters, however, usually vary almost as much as in the wild cultures just analyzed.

Root-tip examinations of hybrids showed the expected number of chromosomes; that is, the sum of the haploid numbers of the parents. Apomixis has been found to occur very rarely in the roses of the Cinnamomeae. Many hundreds of buds have been shorn of everything but the hypanthium and achenes, just before they opened. No fruits have been obtained afterward, other than those originally reported for *R. blanda* (14).

The North American hexaploids are highly fertile and give fertile  $F_1$  hybrids with each other. The  $F_1$  from *R. acicularis*  $\times$  *nutkana*, and the reciprocal, is of lower stature than *R. nutkana*. The stems are armed with scattered bristles and straight infrastipular prickles; the fruits are large (to 2 cm. in diameter) and usually pendant. Such plants in nature are often classified as *R. engelmannii* S. Wats., and this name is retained at present.

*Rosa blanda* gives fertile hybrids with *R. palustris*. Both species are diploids, and their ranges coincide over a large area in the region of the Great Lakes. Most of the  $F_1$  hybrids resemble very closely

*R. schuettiana* Erlanson, a species that has been found in Wisconsin, Michigan, and New York (13). One out of 18  $F_1$  hybrids in one culture resembles *R. michiganensis* Erlanson, which type is also more rare in nature than *R. schuettiana*. *R. palustris* gave a sterile  $F_1$  with *R. foliolosa*, although both are diploid.

*R. blanda* also gives fertile vigorous hybrids with the diploid *R. woodsii*. An intergrading series between these forms makes it hard to classify the diploid roses of the northern Great Plains region. In herbaria these forms are usually called *R. macounii*, a species that is probably a definite ecotype of *R. woodsii*. LUNELL's description of *R. naiadum* Lunell covers this hybrid, which resembles *R. woodsii* and may be used to designate intermediate forms. *R. woodsii* gave partially sterile  $F_1$  hybrids with diploid *R. pisocarpa*.

*R. blanda* and *R. woodsii* both give partially sterile tetraploid  $F_1$  hybrids with *R. acicularis*, which bear corymbs of flowers and fruits.<sup>5</sup> One of these crosses may have originally synchronized with mutations and given rise to the tetraploids of the Great Plains. *R. arkansana* gives a semisterile pentaploid  $F_1$  with hexaploid *R. acicularis*.

The fertile tetraploid  $F_1$  hybrid between *R. arkansana* (4x) and *R. carolina* (4x) is frequent on the eastern Great Plains and gives the species complex  $\times R. rudiusscula$  Greene (17). The tetraploid species *R. carolina* and *R. virginiana* give a fertile  $F_1$  which is intermediate and may be designated for convenience as  $\times R. obovata$  Raf., since the type was collected in the Catskill Mountains, New York, where the parent species are both found. All triploid  $F_1$  hybrids, from diploid crossed with tetraploid species, are of course highly sterile. The hypanthia usually all abort after anthesis, although hips and achenes have been observed on some plants of *R. blanda*  $\times$  *virginiana*.

Although hybridization is thus known to be taking place in nature, it is nevertheless possible to recognize some of the  $F_1$  types and also to distinguish a few definite Linnaean species. By allowing a wide range of parallel variation to these species, we can ignore later hybrid generations, in which the parent types probably segregate out again.

<sup>5</sup> The one large corymb recorded by CRÉPIN (9) for *R. acicularis* was on a plant collected by DRUMMOND at Cumberland House Fort. This specimen resembles *R. blanda*  $\times$  *acicularis*.

### Nomenclatural treatment of parallel variations

When I tabulated the combinations of characters in the collective species *R. acicularis* (12) and gave new varietal names to some of them, Mr. C. A. WEATHERBY wrote, "I am glad you are not afraid to provide names enough to cover all such combinations, when reasonably clear-cut." Since then it has been found that this is not practicable, for besides the individual variations which are shown in appendix I there are other variables. Stem colors—brown, green, and red—segregate; so do erect and decumbent, strict and subcernuous habits. Glaucous stems or foliage and non-glaucous are found in single cultures. Foliage texture and surface (lustrous or dull), color of filaments, petals and styles, all vary. Resistance to rusts and mildews differs in individuals of the same culture. We may easily have to deal with ten or twelve pairs of contrasting characters that are probably Mendelian, and usually assort independently. The resulting number of possible combinations is large, and hence a system of varietal names becomes unwieldy. Confusion lies this way also, for eventually we should be finding several varieties on the same bush, just as at present we find more than one species.

My firm conviction is that these minor parallel variations belong *sui generis* as to each Linnaean species, and that they can be treated only by using numerical tabulations, as has been done in the tables of appendix I. If any other method be followed, it leads to hopeless nomenclatural confusion, degradation of the species, and the sort of situation that exists in the American species of *Crataegus* (1).

### Linneons in the American Cinnamomeae

As a result of these experiments and conclusions, I would admit only sixteen of the recognized groups of the Cinnamomeae in North America to unquestioned specific rank. Ten of them are collective species, namely: *R. acicularis*, *R. arkansana*, *R. blanda*, *R. californica*, *R. carolina*, *R. durandii*, *R. gymnocarpa*, *R. nutkana*, *R. pisoncarpa*, and *R. woodsii*.

These groups all show a wide variation of habit types, possess geographic races within a wide range of distribution, and exhibit parallel variation. Once these phenomena are admitted, the species or Linneons can be distinguished from one another in the field, after

a little experience. They can often be identified from good herbarium material with the help of general characteristics and numerical variations. In table I, *R. blanda*, *R. californica*, *R. pisocarpa*, and *R. woodsii* are compared with regard to numerical variations of certain characteristics. In the accompanying analytical key (appendix III) an attempt has been made to employ and stress those characteristics which have been found not to occur as parallel variations. Chromosome number is an important characteristic and is given for each species in the key.

Species that are relatively stable and that can usually be recognized in the field and herbarium are: *R. foliolosa*, *R. nitida*, *R. palustris*, *R. rugosa*,<sup>6</sup> *R. spithamea*, and *R. virginiana*.

Among these 16 species, five have a distribution that is chiefly eastern on this continent, nine are chiefly western, and only two, *R. arkansana* and *R. foliolosa*, are centrally distributed. This is interesting and logical when the geological history of the North American continent is taken into consideration.

Of the six F<sub>1</sub> hybrid species, only four ( $\times$  *R. engelmannii*,  $\times$  *R. michiganensis*,  $\times$  *R. schuettiana*, and  $\times$  *R. rudiusscula*) have been included in the key.  $\times$  *R. obovata* and  $\times$  *R. naiadum* closely resemble *R. carolina* and *R. woodsii* respectively.

#### ECOTYPE SPECIES

There are also seven groups that I now retain tentatively in specific rank which are suspected of being ecotypes (40) of various collective species.

*R. williamsii* Fernald, known only at Bic, Quebec, may be a caliphile ecotype of *R. blanda*.

*R. macounii* Greene, from GREENE's description, resembles a compact form of *R. woodsii* with 7-11 small leaflets which I have obtained from Nebraska, South Dakota, and Saskatchewan. GREENE

<sup>6</sup> A specimen of *R. rugosa* Thunb. (9645) from seed collected near Wrangell, Alaska, by DR. A. S. WARTHIN in 1926 was raised at Ann Arbor. This is the more slender form with elliptical leaflets, which is given by REHDER (32) as *R. kamtschatica* Vent., a synonym of *R. rugosa*. More material of this species should be searched for in Alaska. This is the first record for an (apparently) endemic specimen of the *R. rugosa* group on the American continent.

says that his species "belongs to the region of dry elevated plains and is sub-alpine." LUNELL named a similar form *R. subnuda*.<sup>7</sup>

*R. alcea* Greene (syn. *R. subglauca* Rydb.) is a dwarf tetraploid which grows in the northern part of the range of *R. arkansana*, on the prairies of Canada. A plant that came from Craigmyle, Alberta, answering to the description of *R. alcea*, has grown at Ann Arbor (3492) for eight years and is only 2 dm. tall (fig. 19). It has never produced any flowering turions, but resembles *R. arkansana* in number of chromosomes, stamens, and teeth on the leaflets. It is suspected of being a northern ecotype of that species.

*R. housei* Erlanson, as already explained, appears to be an eastern swamp-inhabiting ecotype or ecophene of *R. arkansana*.

*R. manca* Greene (syn. *R. aciculata* Cockerell, *R. pecosensis* Cockerell) is a dwarf hexaploid of the southern Rocky Mountain region, closely allied to *R. nutkana*.

*R. yainacensis* Greene<sup>8</sup> (syn. *R. pinetorum* Heller, *R. myriadenia* Greene) is a low tetraploid form of dry habitats in the southern part of the range of *R. durandii* Crépin. It is more glabrous and has not been found with puberulent stems, but is judged to be closely related to, and perhaps a southern ecotype of, that species.

*R. calvaria* Greene is a diploid closely related to *R. gymnocarpa*, as GREENE also knew. The calyx is caudate attenuate and is not deciduous; RYDBERG therefore classified it with *R. pinetorum* (*R. yainacensis*), which, however, is tetraploid. Root tips from a plant of *R. calvaria* from Tuolumne Big Tree Grove, Tuolumne County, California, showed 14 somatic chromosomes. It is here retained as a questionable ecotype of *R. gymnocarpa* belonging to the southern Sierra Nevada. It is hoped that cultures will be grown in California to test this form,<sup>9</sup> as well as the interesting low growing tetraploids *R. yainacensis* and *R. spithamea*.

<sup>7</sup> RYDBERG overlooked GREENE's description and called pubescent variants of *R. woodsii* by this name. These were described as *R. grosseserrata* by NELSON, but NELSON's name is not in my opinion synonymous with *R. macounii* or *R. subnuda*.

<sup>8</sup> Living material was obtained from Fort Ross, Calif., and from Pacific Grove, Monterey Bay, the type locality of *R. pinetorum*. Herbarium material shows that *R. yainacensis* and *R. pinetorum* are essentially the same, and the former name has priority.

<sup>9</sup> *R. bolanderi* Greene and *R. covillei* Greene were also reported by GREENE to belong to the group of *R. gymnocarpa* and to possess short ovate non-deciduous sepals. This characteristic is not sufficient for specific distinction from *R. gymnocarpa*, and these two "species" are considered to be merely minor variants.

The octoploid form of *R. acicularis* is probably the type form since a hexaploid has not been reported for Europe. CRÉPIN (10) suggested that the American form be separated as *R. sayi* Schw. On the evidence of flowering time and stamen number (table II; fig. 2), it would be justifiable to call the hexaploid *R. acicularis* var. *sayi* (Schw.) Rehder, at least for horticultural use. The two forms cannot be distinguished in herbarium material and the distribution of the octoploid needs to be worked out.

In appendix II there are listed alphabetically the 16 Linnaean species that are now retained, together with the ecotype species, if any, and synonyms related to each from the evidence of parallel variation. The synonyms include the 110 species given by RYDBERG (34) for the sections *Carolinae*, *Cinnamomeae*, and *Gymnocarpae*, plus five species listed by him as synonyms for *R. macounii*, plus three species described by the writer (13). A brief preliminary account of these conclusions was previously published (20).

### Summary

1. Herbarium, field, and garden studies upon extensive series of North American wild roses belonging to the section *Cinnamomeae* have shown that many of the characteristics commonly used to distinguish between species are individual variations. These unreliable characteristics may occur combined in every possible way, sometimes in plants of a single culture. They also appear in every species and give good series of parallel variations. Because in the past rhodologists have been inclined to give these minor variations and their multitudinous combinations specific or varietal rank, nomenclatural confusion has resulted.

2. The various diagnostic characteristics are discussed and evaluated chiefly from evidence obtained from growing cultures of plants raised from the seeds of single wild individuals. As many as ten so-called species have appeared in one culture.

3. The offspring of isolated diploid plants of *R. woodsii* from the Great Basin of Utah showed as much variability and pollen sterility as offspring of plants of *R. blanda* from northern Michigan, which grew close to roses belonging to other species groups. Hybridization is not therefore the most important source of variation.

4. Tables are presented which demonstrate the variation in minor



characteristics in cultures of the groups of *R. blanda*, *R. californica*, *R. pisocarpa*, and *R. woodsii*.

5. On the evidence of data from these and other cultures, the sections Carolinae and Gymnocarpae have been merged with the section Cinnamomeae. One hundred ten species in this section given by RYDBERG (34) and three species described by the writer have been placed in 16 Linnaean species. These 16 species are relatively stable (although individuals are highly heterozygous) and they can be distinguished from one another by general, morphological, numerical, cytological, and physiological characteristics. Ten of the sixteen are collective species with wide ranges of distribution.

6. Seven other species are suspected of being ecotypes of the various collective species but have been retained in specific rank.

7. Interspecific  $F_1$  hybrids have been raised after crossing species with ranges that overlap. Several of these hybrids are fertile and resemble roses that occur in nature. Six previously described species are considered on this evidence to be  $F_1$  hybrids.

8. These results which attribute a few highly variable Linnaean species to the genus *Rosa* agree with those obtained by BOULENGER from data from European rose species.

9. Sterility arises from (a) interchange, (b) internal polyploidy, (c) hybridization with polyploid species. Polyploidy is therefore an obstacle to the fusion of species.

10. Ninety-one specific names, chiefly from RYDBERG (34), have been given as synonyms in an alphabetical list of Linneons. An analytical key is appended which includes 16 Linnaean species, seven ecotype species, and four hybrid species in the section Cinnamomeae.

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## Appendix I

## Key to symbols used for parallel variations in cultures

## B Bristles

- o absent
- 1 basal
- 2 to twig tips
- 3 deflexed
- 4 glandular
- 5 pubescent

## P Prickles

- o absent
- 1 straight
- 2 terete
- 3 deflexed
- 4 flattened
- 5 curved
- 6 ascending

## L Leaflet shape

- 1 oval
- 2 elliptical
- 3 ovate
- 4 obovate
- 5 cordate base
- 6 orbicular

## Lsc Leaflet surface covering

- o glabrous
- 1 appressed pubescent, both surfaces
- 1v villous, both surfaces
- 2 appressed pubescent beneath
- 2v villous beneath
- 3 pubescent on veins beneath
- 3v villous on veins beneath
- 4 gland-muriculate
- 5 gland-muriculate on veins below
- 6 gland-pruinose
- 7 glandular-granuliferous

## R Rhachis

- o glabrous
- 1 pubescent
- 2 gland-pruinose
- 3 gland-hispid
- 4 setaceous

## T Teeth on leaflets

- 1 simple
- 2 bidentate
- 3 serrulate
- 4 gland-serrulate

## H Hypanthium in fruit

- o glabrous
- 1 globose
- 2 depressed globose
- 3 with neck (urceolate)
- 4 pyriform
- 5 ellipsoid
- 6 pilose
- 7 gland-hispid
- 8 hispid

## S Sepals

- o glabrous
- 1 puberulent on edge
- 2 puberulent on back
- 3 gland-hispid on edge
- 4 gland-hispid on back
- 5 reflexed
- 6 spreading
- 7 erect
- 8 connivent
- 9 deciduous

## F Pedicel

- o glabrous
- 1 puberulent
- 2 pilose
- 3 gland-hispid
- 4 glandular-granuliferous

## VARIATION IN OFFSPRING OF SINGLE WILD INDIVIDUALS

TABLE A

COLLECTIVE SPECIES R. BLANDA

CULTURE 3753/7 (56 OFFSPRING\*). PARENT: R. BLANDA VAR. GLANDULOSA, MACKINAC CO., MICHIGAN. HIPS ALL GLABROUS AND SMOOTH

B	P	L	Lsc	R	T	H	S	IDENTIFICATION ACCORDING TO RYDBERG'S KEY
Parent								
I	o	I	I2V	I	I	5	47	blanda var. glandulosa
Offspring								
I	o	I	I2V	I	4	5	47	acicularioides
2	o	I	I2V	I	I	I	47	blanda var. hispida
I	o	I-4	I2V	I	I	I	37	blanda
I	o	I	I2V	I	I	I	46	blanda
2	o	I-2	I2V	4	I	I	46	blanda
I	o	I	I2V	I	I	I	47	blanda
I	o	I-2	I2V	I	I	I	47	blanda
I	o	I	I2V	I	I	4	07	blanda var. glandulosa
I	o	I	I	I	I	3-4	47	blanda var. glandulosa
I	o	I	I2V	4	I	I-5	47	blanda var. glandulosa
2	o	I-2	2	I	I	I-4	07	blanda var. glandulosa
2	o	I-4	2	I	I	.....	.....	Dwarf
2	o	I	I2V	I	I	I-4	37	blanda var. glandulosa
I	o	I	2	4	I	I	46-7	blanda
I	o	I	I2V	I	I	I-4	36	blanda
I	o	I-4	I2V	I	I	I-4	46	blanda
I	o	I	I2V	I	I	I	46-7	blanda
2	o	I-4	2	I	I	.....	.....	Dwarf
I	o	I-2	I2V	I	I	I-2	06	blanda
I	o	I-4	2	4	I	I	47	blanda
2	o	I	I2V	I	2-4	2	07	acicularioides
I	o	I	I2V	I	I	I	07	blanda
I	o	I-2	2	I	I	I	36	blanda
I	o	I	I2V	4	I-2	I	47	blanda
I	o	I-4	I2V	I	I	I-4	47	blanda
I	o	I	I	I	I-2	I	46	blanda
I	o	I	I	I	I	I-2	47	blanda
I	o	I	I2V	4	I	I	47	blanda
I	o	I	I	I	I	3	06-5	blanda var. glandulosa
I	o	I	I2V	I	I	3	35	blanda var. glandulosa
I	o	I	I2V	I	I	I	47	blanda
I	o	I-2	2	I	I	2	47	blanda
2	o	I-4	I2V	I	I-4	I	46	acicularioides
I	o	I	2	I	I	I	37	blanda
I	o	I	2	I	I	I	36	blanda
I	o	I-2	I2V	4	I	I	47	blanda
2	o	3-6	2	4	I	.....	.....	Dwarf
I	o	I-2	I2V	I	I	5	47	blanda var. glandulosa
I	o	I	I2V	I	I	4	47	blanda var. glandulosa
I	o	I	I	I	I-4	I	07	blanda
2	o	I-4	2	I	I	.....	3	blanda (sterile triploid)
2	o	I	2	4	I	I	47	blanda
2	o	I-4	2	I	I	4	46	blanda var. glandulosa

TABLE A—Continued

B	P	L	Lsc	R	T	H	S	IDENTIFICATION ACCORD- ING TO RYDBERG'S KEY
Offspring —Con- tinued								
I	o	I	12V	I	I	I	47	blanda
I	12	I	12V	I	I	4	37	blanda var. glandulosa
I	o	I-2	3	I	I	2	36	blanda
I	o	I	12V	I	2-4	I	07	blanda
I	o	I	o	I	I	2	47	subblanda
2	o	I-4	2	4	I	5	46	blanda var. glandulosa
2	o	I-4	12V	I	I-4	.....	.....	Dwarf
I	o	I	12V	I	I	2	46	blanda
2	o	I	12V	I	I	I	07-6	blanda var. hispida
2	o	2	o	4	I	.....	.....	subblanda (dwarf)
I	2-5	I	12V	I	I	I	07	palustriformis
2	o	I-2	12V	I	I	I	07	Sterile tetraploid dwarf

\* Stem color: reddish brown, 46; greenish brown, 10.

TABLE B

## COLLECTIVE SPECIES R. BLANDA (CONTINUED)

CULTURE 12325 (10 OFFSPRING). PARENT: R. BLANDA, GASPÉ PENINSULA, QUEBEC. ALL PLANTS WITHOUT INFRASTIPULAR PRICKLES AND WITH 5-9 OVAL LEAFLETS AND SIMPLE TEETH. FORMULAE GIVEN FOR OFFSPRING ONLY

B	Lsc	R	H	S	IDENTIFICATION
I	I	I	I-27	46-7	blanda var. carpohispida
2	I	3	20	36-7	blanda
I	I	3	10-7	47	blanda var. carpohispida
I	o	o	10	37	subblanda
I	I	3	I-47	46-7	blanda var. carpohispida
I	I7	I	10	46-7	blanda
I	2	I	I-50	06-7	blanda var. glandulosa
2	I	3	10	47	blanda
I	o	o	10	36-7	subblanda
I	I	I	27	07	blanda var. carpohispida

TABLE C

COLLECTIVE SPECIES *R. WOODSII*\*

CULTURES 12191 (9 OFFSPRING) AND 12193 (1 OFFSPRING). PARENT: *R. MACOUNII*, SALT LAKE CITY, UTAH. ALL PLANTS BRISTLY AT BASE, AND WITH GLOBOSE GLABROUS HIPS

P	L	Lsc	R	T	H	S	IDENTIFICATION
12191							
Parent							
12	1-2	1	1	1	2	17	macounii
Offspring							
32	1	1	1	1	1	17	puberulenta
0	1	1	1	1	1	.....	Dwarf
12	1	0	0	1-2	1	17	woodsii
32	3-2	17	1	1-2	1	07	puberulenta
45	1-3	0	14	1	1	27	woodsii
12	1-4	17	1	1	2	07	macounii
0	4-2	17	1	1	1	27	salicetorum
12	4-2	17	1	4	2	27	fendleri
32	1-2	1	1	3-4	1	17	granulifera
12193							
Parent							
12	1-2	1	1	1	1	06	macounii
Offspring							
15	1-4	18	12	1	1	07	puberulenta

\* Culture 12211 (9 offspring). Parent: *R. macounii*, Reno, Nevada. Height of offspring: over 1 m., 7 plants; dwarfs (under 0.5 m.), 2 plants. Badly rusted, 3 plants. Stems: green in 5 plants, brown in 1 plant, red in 4 plants, glaucous in 2 plants. According to RYDBERG's key the plants would be classified from leaf characters as: *R. fendleri*, 3 plants; *R. macounii*, 6 plants. Only 2 flowered in 1931 and both had 50% of the pollen shriveled.

Culture 12220 (38 offspring). Parent: *R. pyrisfera*, Reno, Nevada. Height: about 1 m., 33 plants; less than 5 dm., 5 plants. Stem color: dull brown, 36 plants; red, 2 plants; glaucous, 15 plants; badly rusted, 9 plants. According to leaf characters, by RYDBERG's key the offspring would be classified as: *R. macounii*, 32 plants; *R. fendleri*, 5 plants; *R. hypoleuca*, 1 plant; *R. fendleri*, 1 plant.

TABLE D

## COLLECTIVE SPECIES R. WOODSII

CULTURE 12205 (21 OFFSPRING). PARENT: R. MACOUNII, SOUTH OF SPARKS, WASHOE COUNTY, NEVADA. PARENT 2.5 M. TALL. ALL PLANTS WITH BRISTLES AT BASE AND GLABROUS HIPPS

B	P	L	Lsc	T	H	S	IDENTIFICATION
Parent							
I	12	2-3	17	1-2	5	16	macounii
Offspring							
I	12	1-2	17	1-3-4	3	05	macounii (semisterile with multivalents 2n=14)
I	12	1-2	17	I	4	25	arizonica (?)
I	12	1-2	17	1-2	.....	.....	.....
I	14	I	I	I	1-4	17	macounii (semisterile with multivalents 2n=14)
I	12	3	0	1-2	.....	.....	Dwarf
I	16	2	27	I	5	16	macounii (semisterile)
2	0	2-4	17	1-2	5	06	macounii (semisterile)
I	12	1-2	27	1-3-4	5	17	fendleri
I	12	2	17	1-3	I	25	fendleri
2	0	1-2	I	I	.....	.....	Dwarf, badly rusted
2	I	I	17	1-2-3	I	05	fendleri
I	12	I	I	I	I	06	macounii
2	0	1-4	17	I	I	16	macounii
2	0	2	17	1-3-4	.....	.....	fendleri
2	12	16	17	I	.....	0	macounii (2n=21, sterile)
I	12	1-3	I	I	I	15	macounii
2	12	1-3	17	1-3-4	I	15	fendleri
2	12	I	16	1-4	.....	.....	fendleri
I	12	16	06	1-2	I	07	hypoleuca
I	12	2-4	17	I	35	17	standleyi
2	12	1-2	36	1-4	I	17	hypoleuca



TABLE E

## COLLECTIVE SPECIES R. WOODSII

CULTURE 12207 (11 OFFSPRING\*). PARENT: R. FENDLERI, SOUTH OF SPARKS, WASHOE CO., NEVADA. ALL PLANTS 1-1.5 M. WITH WEAK SCATTERED BRISTLES AND FEW TERETE PAIRED PRICKLES

L	Lcs	R	T	H	S	IDENTIFICATION
Parent						
I	17	12	1-4	I	46	fendleri
Offspring						
I	07	0	1-4	.....	.....	hypoleuca
I	17	12	1-4	.....	.....	fendleri
2	16	12	I	5	15	fendleri
2	16	12	I	.....	.....	fendleri
I	36	12	1-4	I	236	fendleri (resin-scented)
I	0	2	I	I	06	woodsii
1-3	0	2	1-3	1-5	05-6	woodsii
I	0	2	1-3	I	25	woodsii
I	0	2	I	I	16	woodsii
I	0	2	I	.....	.....	woodsii
6	0	2	I	.....	.....	woodsii

\* Highly homozygous for this group. Pollen sterility low (5-12%) in four plants (see table III).

TABLE F

## COLLECTIVE SPECIES R. WOODSII

CULTURE 12209 (52 OFFSPRING). PARENT: R. PYRIFERA, SPARKS, WASHOE CO., NEVADA. OFFSPRING 0.3-2 M. HIGH WITH STEMS BRISTLY AT BASE, SCATTERED BRISTLES AND WEAK PAIRED PRICKLES ON TWIGGS. STEMS REDDISH. LEAFLETS BROADLY OVAL, YELLOWISH GREEN. MANY RUSTED. FEW FLOWERS; SEPALS ERECT TO REFLEXED

B	P	Lsc	R	T	H	S	IDENTIFICATION
Parent							
I	12	3	2	I	4-5	I	pyrifera
Offspring							
I	12	0	0	I	.....	.....	woodsii
I	12	06	2	I-4	I	15	hypoleuca
I	12	26	2	I	.....	.....	fendleri
I	12	26	2	I	.....	.....	fendleri (rusted)
I	12	27	2	I	.....	.....	.....
2	0	07	2	I	.....	.....	Dwarf (15 cm.)
2	0	27	2	I	.....	.....	Dwarf (8 dm.)
2	12	2	I	I	I	045	gratissima (?)
I	12	0	I	I	.....	.....	woodsii
I	13	2	4	I	.....	.....	macounii
I	13	2	4	I	.....	.....	macounii
2	0	26	2	I	.....	.....	fendleri (dwarf, 8 dm.)
2	12	26	2	I	I	06-7	gratissima
2	13	27	4	I-2	13	14	gratissima (2n=14, sterile)
I	0	I	I	I	.....	.....	.....
2	12	3	I	I	.....	.....	woodsii
2	12	27	2	I	I	15	macounii
I	12	0	2	I	.....	.....	woodsii
I	12	2	I	I	.....	.....	macounii
2	0	0	2	I	.....	.....	woodsii
2	0	0	2	I	.....	.....	gratissima
2	12	27	2	I	5	0	pyrifera
2	12	06	2	2-4	.....	.....	hypoleuca
2	0	06	2	2-4	.....	.....	hypoleuca (rusted)
2	12	0	0	I	.....	.....	woodsii (rusted)
2	12	2	I	I	4	0	pyrifera
2	0	2	I	I	.....	.....	.....
2	12	2	I	I	.....	.....	.....
2	12	07	2	I	.....	.....	hypoleuca
2	12	2	I	I	.....	.....	.....
2	0	2	I	I	.....	.....	.....
2	0	2	I	I	I	146	gratissima
2	12	0	0	I-4	.....	.....	hypoleuca
2	0	2	I	I	.....	.....	..... (badly rusted)
2	12	17	2	I	.....	.....	fendleri (rusted)
2	12	17	2	I	.....	.....	fendleri
2	12	07	2	I	.....	.....	hypoleuca
2	12	2	I	I	.....	.....	gratissima (badly rusted)
2	0	27	2	I	.....	.....	gratissima
I	12	27	2	I	.....	.....	gratissima

TABLE F—*Continued*

B	P	Lsc	R	T	H	S	IDENTIFICATION
2	0	0	2	4	.....	.....	hypoleuca (rusted)
2	0	27	2	1	.....	.....	gratissima
2	0	27	2	1-2	3	2	gratissima
2	12	27	2	1	.....	.....	.....
2	0	27	2	1	.....	.....	.....
2	0	0	2	1	.....	.....	woodsii (rusted)
2	0	2	2	4	.....	.....	fendleri (rusted)
2	0	27	2	1	.....	.....	gratissima
2	0	27	2	1	.....	.....	2n = 21
2	12	27	2	1	1	2	gratissima
2	0	0	0	1	.....	.....	woodsii
2	12	0	0	1	.....	.....	woodsii

TABLE G

## COLLECTIVE SPECIES R. WOODSII

CULTURE 12212 (2 OFFSPRING). PARENT: R. PUBERULENTA, RENO, NEVADA

B	P	L	Lsc	T	H	S	IDENTIFICATION
Parent							
1	13	1	2	1	1-4	27	puberulenta
Offspring							
2	0	1-3	1	1	.....	.....	macounii
13	13	1-2	27	1	4	27	neomexicana (5 ft. tall; bad pollen, 3%)

TABLE H  
COLLECTIVE SPECIES *R. WOODSII*

CULTURES 12213 (4 OFFSPRING) AND 12218 (6 OFFSPRING). PARENT: *R. SALICETORUM*, RENO, NEVADA

B	P	L	Lsc	T	H	S	IDENTIFICATION
12213 Parent							
1	o	1	17	1	4	17	salicetorum
Offspring							
2	o	1-2	17	1	.....	.....	macounii (rusted)
1	o	2	17	1	1	15	salicetorum (rusted)
2	o	2	27	1	1	16	macounii
2	12	1-2	27	1-2	.....	.....	macounii
12218 Parent							
1	o	1	17	1	1	1	salicetorum
Offspring							
2	o	1	07	1-4	.....	.....	hypoleuca
2	o	1	17	1-2-4	.....	.....	fendleri
2	o	1	1	1	.....	.....	macounii
2	o	2	17	1-2	.....	.....	fendleri
2	o	1	17	1	.....	.....	macounii
2	o	1	17	3	.....	.....	macounii

TABLE I  
COLLECTIVE SPECIES *R. WOODSII*

CULTURE 12220 (38 LIVING OFFSPRING,\* DATA IN TABLE ONLY FOR 12 THAT FLOW-ERED). PARENT: *R. PYRIFERA*, RENO, NEVADA. ALL PLANTS WITH BRISTLES AT BASE AND STRAIGHT TERETE PRICKLES

L	Lsc	T	H	S	IDENTIFICATION
Parent					
1	2	1	4	17	pyrifera
Offspring					
1	17	1	4	17	pyrifera
1	26	1-4	1	17	fendleri
1	2	1	1	17	macounii
1	27	1	1	16	macounii
1-2	2	1	1	17	macounii
1	27	1	4	17	macounii
1	06	1-4	1	17	hypoleuca
1	2	1	1	17	macounii
1	37	1	1	16	macounii
1	27	1	1	17	macounii
1	2	1	1	16	macounii
1-2	2	1	1	16	macounii

\* General segregation of characters among 38 plants. Height; about 1 m., 33 plants; about 0.5 m., 5 plants. Badly rusted, 9. Stem color: dull brown, 36; reddish, 2; glaucous stems, 15. Leaf types: "*R. macounii*," 32 plants; "*R. woodsii*," 1; "*R. fendleri*," 1; "*R. hypoleuca*," 1.

TABLE J

COLLECTIVE SPECIES *R. WOODSII*

CULTURE 12231 (3 OFFSPRING). PARENT: *R. GRANULIFERA*, PYRAMID LAKE, NEVADA\*

B	P	L	Lsc	T	H	S	IDENTIFICATION
Parent							
23	25	1	26	4	1	17	granulifera
Offspring							
2	12	1-2	37	1	.....	.....	gratissima
23	23	2-3	27	1-4	1	16	granulifera
2	12	1	27	1	.....	.....	gratissima

\* Parent plant isolated in sagebrush plains and desert. No other roses for 40 miles around. All 3 plants rusted.

TABLE K

COLLECTIVE SPECIES *R. PISOCARPA*

CULTURE 12248 (4 OFFSPRING\*). PARENT: *R. PISOCARPA*, MT. SHASTA CITY, CALIF. ALL PLANTS: HABIT STRICT, HEIGHT 1-1.5 M; A FEW BRISTLES AT BASE AND A FEW WEAK, TERETE INFRASTIPULAR PRICKLES; HYPANTHIUM GLABROUS AND GLOBOSE, 7-9 MM. DIAM.

L	Lsc	T	S	IDENTIFICATION
Parent				
1-3	0	1	4	pisocarpa
Offspring				
1-3	1	1	.....	.....
2	3	1	15	ultramontana
1-3	17	1	15	ultramontana
1-3	2	1	46	pisocarpa

\* Stems: green, 2; reddish, 2. All plants rusted.

TABLE L

COLLECTIVE SPECIES *R. PISOCARPA*CULTURE 12259 (1 OFFSPRING). PARENT: *R. PRINGLEI*, JACKSONVILLE, OREGON

B	P	L	Lsc	T	H	S	IDENTIFICATION
Parent ○	○	1-3	3	1	1-5	37	pringlei
Offspring ○	○	1-2-3	2	1	.....	3	anacantha (triploid)

TABLE M

COLLECTIVE SPECIES *R. PISOCARPA*CULTURES 12262 (1 OFFSPRING) AND 12268 (1 OFFSPRING). PARENT: *R. COPELANDII*, ASHLAND CAÑON, OREGON

B	P	L	Lsc	T	H	S	IDENTIFICATION
12262 Parent 1							
Offspring 2	12	3	○	1	5	37	copelandii
12268 Parent 2	12	3	3	2-4	.....	.....	.....
Offspring ○	1	3	○	1	5	37	copelandii
	○	2-3	○	1	3	37	copelandii

TABLE N  
COLLECTIVE SPECIES *R. PISOCARPA*

CULTURE 12280 (25 OFFSPRING,\* DATA IN TABLE ONLY FOR 13 THAT FLOWERED);  
PARENT: *R. PISOCARPA*, CORVALLIS, OREGON. ALL STEMS GREENISH, GLAUOUS.  
ALL LEAFLETS GLAUOUS BELOW

B	P	L	Lsc	T	H	S	IDENTIFICATION
Parent							
o	26	35	2	I	I	16	<i>pisocarpa</i>
Offspring							
o	o	35	2	I	I	47	<i>anacantha</i>
o	o	I	2	I	I	46-9†	<i>anacantha</i>
I	o	35	2	I	I	46	<i>pisocarpa</i>
I	o	35	2	I	I	47	<i>pisocarpa</i>
I	o	I-35	3	I	I	10-46	<i>pisocarpa</i> ‡
o	26	35	3	I	3	10-3	<i>pringlei</i> ‡
I	o	35	3	I	I	47	<i>pisocarpa</i>
I	o	35	3	I	I	36-9†	<i>pisocarpa</i>
o	26	I-35	3	I	I	36	<i>pisocarpa</i>
I	o	35	2	I	I	47	<i>pisocarpa</i>
o	26	15	2	I	3	46	<i>pringlei</i>
o	0-26	35	2	I	I	47	<i>pisocarpa</i>
o	26	35	2	I	I	47	<i>pisocarpa</i>

\* Bristles: none, 17; basal, 8. Prickles: none, 20; terete and ascending, 5. Stems: slender, with arched branches, except 3 plants. One plant leaflets orbicular, cordate at base (22×20 mm.; 11×10 mm.).

† Note partially deciduous sepals.

‡ RYDBERG gives eglandular sepals to *R. pringlei* and *R. copelandii*, and insists upon glandular-hispid sepals for *R. pisocarpa*.

TABLE O  
COLLECTIVE SPECIES *R. PISOCARPA*

CULTURE 12284 (21 OFFSPRING,\* DATA IN TABLE ONLY FOR 9 THAT FLOWERED).  
PARENT: *R. PRINGLEI*, SOUTH OF CORVALLIS, OREGON. RARELY A FEW BRISTLES  
AT BASE

B	P	L	Lsc	T	H	S	IDENTIFICATION
Parent							
o	0-26	3	2	I	35	46	<i>pringlei</i>
Offspring							
o	26	3	2	I	I	47	<i>pisocarpa</i>
I	26	3	2	I	13	46	"
I	26	3	2	I	I	47	"
I	26	I	3	I	13	45-6	"
o	26	3	2	I	I	47	"
I	26	I	2	I	13	45	"
o	26	3	2	I	I	47	"
o	o	3	2	I	13	47	<i>anacantha</i>
I	26	3	3	I	I	47	<i>pisocarpa</i>

\* Stems 0.9-2 m., 1 dwarf (4 dm.); glaucous red-brown (18 plants), greenish (3 plants); arched (18 plants), strict (3 plants); few slender ascending paired prickles (10 plants), completely unarmed (2 plants). Leaflets ovate to oval, base usually somewhat cordate, pale glaucous and finely appressed puberulent beneath. Rachis gland-hispid in 2 plants. Formula of dwarf, BO P26 Lsl Lcs2 T4.

TABLE P

COLLECTIVE SPECIES *R. CALIFORNICA*

CULTURE 12295 (4 OFFSPRING\*). PARENT: *R. CALIFORNICA*, CASHION CAÑON, CONTRA COSTA CO., CALIF.

B	P	L	Lsc	T	H	S	F	IDENTIFICATION
Parent I								
Offspring I	34	13	IV	I	13	2	2	californica
I	0	13	IV7	I	I	46	2	johnstonii
I	14	23	IV	I	4	26	2	johnstonii
2	0	13	IV	I	.....	.....	.....	Dwarf
I	12	23	IV	I	4	26-7	2	johnstonii

\* Habit: arched, 3; strict, 1; dwarfs, 1. Stems: brown, 3; green, 1; glaucous, 1.

TABLE Q

COLLECTIVE SPECIES *R. CALIFORNICA*

CULTURE 12303 (5 OFFSPRING\*). PARENT: *R. CALIFORNICA*, BERKELEY, CALIF. STEMS ALL RED-BROWN. PRICKLES VARIABLE EVEN ON SAME BUSH. HYPANTHIA AND PEDICELS PILOSE

B	P	L	Lsc	T	H	S	IDENTIFICATION
Parent I							
Offspring 2	25	I	IV2	I	I	17	californica
2	23-5	I-6	2V7	I	I	27	californica
2	23-5	I-6	2V7	I	I	23	californica
I	12	I-6	IV	I	.....	.....	johnstonii
2	16	I	IV	4	.....	.....	bidenticulata (dwarf)
2	14	I	IV7	I	I	26	johnstonii

\* Habit: arched, 3; strict, 1; dwarfs, 1.



TABLE R

## COLLECTIVE SPECIES R. CALIFORNICA

CULTURE 12307 (44 OFFSPRING). PARENT: R. SANCTAE-CRUCIS, REDHILL, CALIF. COARSE STURDY PLANTS. STEMS 1-1.5 M., MUCH BRANCHED, ARCHING TO GROUND. LEAFLETS 5-7 (RARELY 9). FLOWERS CORYMBOSE. STIGMATIC HEADS FLAT TO 3 MM. HIGH

B	P	L	Lsc	T	H	S	F	IDENTIFICATION
Parent								
I	14	I-3	IV7	I-4	13	25	2	sanctae-crucis
Offspring								
I	45	I-3	IV7	I	2	16	0	brachycarpa (stigmatic head raised 3 mm.)
2	I-32	I-2	IV	I	I	25	2	johnstonii
I	12	I-6	IV	I	I	26	24	johnstonii
I	I-34	I-4	IV	I	3	26	2	johnstonii
I	14	I-3	IV	I	I	25	23	davyi
2	12	I-3	IV7	3-4	I	27	2	?corymbiflora (resin-scented)
2	12	I	IV7	I-4	I	27	2	?corymbiflora
I	2-43	I	IV7	I-4	I	237	3	breweri (stem under flowers glandular-hispid)
2	21	I-3	IV	I	I	2	2	johnstonii
2	I-32	I-2	IV	I	I	25	2	johnstonii
I	45	I-3	IV	I	I	2	2	californica
I	14	I-6	IV	I	I	2	13	davyi
2	12	I-4	IV	I	I	26	2	johnstonii
I	45	I-3	IV7	I	2	16	0	californica
I	14	I-6	IV7	I	I	247	3	sanctae-crucis
2	12	I	IV7	4	I	247	3	corymbiflora
2	12	4-6	IV	I	I	27	0	johnstonii
2	12	I	IV	I	3	27	0	johnstonii
I	I2-4	4-6	IV	I	I	2	23	johnstonii
2	I2-4	I	I7	I	I	27	0	johnstonii
I	I2-4	I-2	IV	I	I	2	2	johnstonii
I	45	I	IV7	I	I	2	2	brachycarpa (stigmatic head raised 3 mm.)
2	12	I	27	2-4	I	24	23	?corymbiflora (dwarf 4 dm.)
2	12	I	IV	I	I	2	0	johnstonii
I	12	I-4	IV	I	13	27	0	johnstonii
I	12	I	IV	I	I	527	0	johnstonii (stem under flowers glandular)
2	12	I-3	IV	I	I	2	0	johnstonii
I	14	I	IV7	I	I	27	2	sanctae-crucis
I	I2-3	I	IV7	I	I	2	2	johnstonii (stem under flowers pilose)
I	14	I-3	IV	I	I	27	2	davyi
2	12	I-6	IV7	I-4	I	26	2	bidenticulata
2	12	I	27	I	I	2	0	johnstonii

TABLE R—Continued

B	P	L	Lsc	T	H	S	F	IDENTIFICATION
Offspring —Cont.								
I	12	I-4	IV	I	I	2	0	johnstonii
I	45	I-3	IV	I	13	26	0	californica
I	14	I	IV7	I	I	27	2	sanctae-crucis
I	14	I-6	27	I	I	2	2	sanctae-crucis
I	45	I	IV7	I-4	13	26	2	aldersonii (stem under flowers pilose)
I	45	I	IV	I	13	27	0	californica
2	12	I	I	I	.....	.....	.....	johnstonii (stems weak, 1 m.)
I	12	I-4	IV	I	I	2	2	johnstonii
I	12	I	IV	I	I	2	2	johnstonii
I	12	I	IV	I	I	2	2	johnstonii
I	45	I-5	IV	I	I	26	2	californica
I	43	I-2	IV7	I-2	14	27	4	greenei

TABLE S

## COLLECTIVE SPECIES R. CALIFORNICA

CULTURE 12314 (3 OFFSPRING). PARENT: R. BIDENTICULATA, HESPERIA, CALIF.  
STEMS STRICT; FLOWERS 1-3

B	P	L	Lsc	T	H	S	F	IDENTIFICATION
Parent								
I	12	I-3	3	I-2	I	26	0	bidenticulata
Offspring								
2	12	I-6	07	I-4	I	05	0	bidenticulata
2	12	I-3	3	I	I	06	0	.....
2	12	I-6	27	I-4	I	06	2	bidenticulata (rachis and stipules glandular)

## Appendix II

An alphabetical list of the 16 Linnaean species in the section Cinnamomeae that are considered worthy of specific rank, together with six F<sub>1</sub> hybrid species, follows. Seven ecotype species are placed under their related Linneons.

The rose species given in the North American Flora by RYDBERG (34), also by the writer in *Rhodora* (13), in the sections Carolinae, Cinnamomeae, and Gymnocarpae, are grouped alphabetically under the Linneon in which each is now included. For further synonymy the North American Flora should be consulted.\*

*R. acicularis* Lindl. 1820.  $2n = 56$  or  $42$  (two races)

Synonyms: *bourgeauiana* Crépin, *butleri* Rydb., *collaris* Rydb.

*R. arkansana* Porter, 1874.  $2n = 28$

Ecotype species: *R. alcea* Greene, *R. housei* Erlanson

Syn.: *R. lunellii* Greene (= *R. alcea*), *R. polyanthema* Lunell, *ratonensis* Erlanson, *subglauca* Rydb. (= *R. alcea*), *suffulta* Greene

*R. blanda* Ait. 1789.  $2n = 14$

Ecotype species: *R. williamsii* Fernald

Syn.: *acicularioides* Schuette, *johannensis* Fernald, *palustriformis* Rydb., *subblanda* Rydb.

*R. californica* Schlecht. & Cham. 1827.  $2n = 28$

Syn.: *R. aldersonii* Greene, *bidenticulata* Rydb. (= *R. californica* var. *bidenticulata* (Rydb.) comb. nov.), *brachycarpa* Rydb., *corymbiflora* Rydb., *davyi* Rydb., *delitescens* Greene, *greenei* Rydb., *johnstonii* Rydb., *myriantha* Carr., *pilifera* Rydb., *sanctae-crucis* Rydb.

*R. carolina* L. 1753.  $2n = 28$

Syn.: *deamii* Erlanson, *gemella* Willd., *lyoni* Pursh, *nanella* Rydb., *palmeri* Rydb., *petiolata* Rydb., *serrulata* Raf., *texarkana* Rydb., *treleasii* Rydb.

*R. durandii* Crépin, 1875.  $2n = 28$

Ecotype species: *R. yainacensis* Greene

Syn.: *muriculata* Greene (in part), *myriadenia* Greene (= *R. yainacensis*), *pinetorum* Heller (= *R. yainacensis*)

× *R. engelmannii* S. Wats. 1889.  $2n = 42$

*R. foliolosa* Nutt. 1840.  $2n = 14$

\* Synonyms given by RYDBERG are not included in this list, except those he gave for *R. macounii* Greene, because culture experiments show that one collective species (*R. woodsii*) and an ecotype of the same species (*R. macounii*) are mingled in his designation of *R. macounii*.

*R. gymnocarpa* Nutt. 1840.  $2n = 14$

Ecotype species: *R. calvaria* Greene

Syn.: *bolanderi* Greene, *bridgesii* Crépin, *covillei* Greene, *dasypoda* Greene, *leucopsis* Greene, *oligocarpa* Greene, *prionota* Greene

× *R. michiganensis* Erlanson, 1928.  $2n = 14$

× *R. naiadum* Lunell, 1913.  $2n = 14$

*R. nitida* Willd. 1809.  $2n = 14$

*R. nutkana* Presl, 1851.  $2n = 42$

Ecotype sp.: *R. manca* Greene

Syn. *aleutensis* Crépin,\* *aciculata* Ckll. (= *R. manca*), *brownii* Rydb., *columbiana* Rydb., *macdougali* Holz., *melina* Greene, *muri-culata* Greene (in part), *oreophila* Rydb., *pecosensis* Ckll. (= *R. manca*), *spaldingii* Crépin, *underwoodii* Rydb.

× *R. obovata* Raf. 1820.  $2n = 28$

*R. palustris* Marsh. 1785.  $2n = 14$

Syn.: *dasystema* Rydb., *floridana* Rydb., *lancifolia* Small, *obtusiuscula* Rydb.

*R. pisocarpa* A. Gray, 1872.  $2n = 14$

Syn.: *anacantha* Greene, *copelandii* Greene, *eastwoodiae* Rydb., *pringlei* Rydb., *rivalis* Eastw., *rotundata* Rydb., *ultramontana* (S. Wats.) Heller

× *R. rudiusscula* Greene, 1911.  $2n = 28$

Syn.: *aucuparia* Rydb., *bushii* Rydb., *conjuncta* Rydb., *relicta* Erlanson

*R. rugosa* Thunb. 1784.  $2n = 14$

× *R. schuettiana* Erlanson, 1928.  $2n = 14$

*R. spithamea* S. Wats. 1880.  $2n = ?$

Syn.: *adenocarpa* Greene, *dudleyi* Rydb., *granulata* Greene, *sonomensis* Greene

*R. virginiana* Mill. 1768.  $2n = 28$

Syn.: *R. bicknellii* Rydb.

*R. woodsii* Lindl. 1820.  $2n = 14$

Ecotype sp.: *R. macounii* Greene

Syn.: *adenosepala* Woot. & St., *arizonica* Rydb., *chrysocarpa* Rydb., *fendleri* Crépin, *granulifera* Rydb., *gratissima* Greene, *grosseserrata*

\* *R. aleutensis* Crépin, Bull. Soc. Bot. Belg. 13:41. 1875. This species has not been mentioned in the North American Flora.

*A. Nels.*, *hypoleuca* Woot. & St., *maximilliana* Nees, *maximilliana* Rydb., *mohavensis* Parish, *neomexicana* Ckll., *praetincta* Ckll., *puberulenta* Rydb., *pyrifera* Rydb., *salicetorum* Rydb., *subnuda* Lunell (= *R. macounii*), *terrens* Lunell

### Appendix III

#### Analytical key to the North American species of Cinnamomeae

A. Calyx lobes after flowering usually deciduous. Inflorescence usually glandular.

B. Calyx lobes usually spreading or reflexed in fruit, and falling separately from the disc. Pedicels erect in fruit. Achenes densely hirsute. Habitat, east of 100th meridian west and north to the St. Lawrence basin.

C. Shrubs of bogs and moist shores. Leaflets 7-9, narrow-oblong, acute at each end, finely serrate.

D. Usually 1-2 m. tall, flowering laterals 10 cm. long or more. Inflorescence corymbose.

E. Often armed with flattened infrastipular prickles. No. of teeth on each side of leaf average 26. Stamens over 200. Sepals usually reflexed on the fruit and deciduous. Late flowering

*R. palustris*

$2n = 14$

EE. Almost unarmed, prickles weak. No. of teeth average 19. Stamens 150-180. Sepals usually erect and persistent or tardily deciduous. Flowering between *R. blanda* and *R. palustris*

× *R. schuettiana*

$2n = 14$

DD. Stems low, under 1 m., usually armed with copious reddish bristles. Flowering laterals under 10 cm. long, flowers 1-3 together. Teeth fine, acute, av. no.

16.....*R. nitida*

$2n = 14$

CC. Shrubs of rocky shores, dry uplands, and plains.

F. Stems stout, much branched. Suckers few, rarely

flowering in first season. Leaflets firm and elliptic. Bristles at base and on new shoots, prickles flattened or absent. Teeth 9-30 (av. 14). Stamens 120-155. Newfoundland to eastern Pennsylvania

*R. virginiana*

$2n = 28$

FF. Stems usually slender, often bristly to tips, if stout usually simple; often decumbent or bending after first season. Prickles, if present, usually terete. Many suckers which often flower with terminal corymbs after main flowering period. Highly variable and "weedy" species.

G. Leaflets mostly 7, highly varied in shape and texture. Teeth coarse, 5-21, av. 12. Stamens 65-130, av. 105. Hypanthium glandular or smooth

*R. carolina*

$2n = 28$

GG. Leaflets mostly 9. Hypanthium often glandular.

H. Leaflets elliptic to oval, stems bristly to top, erect and simple, or branched and decumbent. Sepals usually spreading in fruit, tardily deciduous or persistent. Stamens 65-143, av. 115..... $\times$  *R. rudiuscula*

$2n = 28$

HH. Leaflets narrow to linear-oblong, acute, glabrous and lustrous above, coarsely serrate. Low and semi-herbaceous, stems almost unarmed. Habitat, southern Great Plains

*R. foliolosa*

$2n = 14$

BB. Calyx lobes falling as a group together with the disc. Inflorescence 1-3 flowers. Pedicels drooping in fruit. Stamens 55-72. Achenes few, large, glabrous. Habitat, west of Great Plains.....*R. gymnocarpa*

$2n = 14$

## AA. Calyx lobes usually persistent after flowering.

## I. Twigs, prickles, and bristles finely pubescent.

- J. Stems stout, bristly, and prickly, occasionally blooming on the turions. Leaflets large, 2-4 cm., elliptic or narrow-obovate, thick, rugose above. Corymbs small. Fruit usually pendent. Foliage eglandular.....*R. rugosa*

2n = 14

- JJ. Plants of Pacific Coast region of U.S.A. Habit varied, 0.3-1.6 m., usually strict. Stems bristly, prickly, and sometimes glandular-hispid. Leaflets small (usually under 2.5 cm.), broadly oval to orbicular, often glandular-muriculate and resin-scented. Inflorescence 1-3. Infra-stipular prickles alate, flattened to terete....*R. durandii*

2n = 28

## II. Twigs, prickles, and bristles glabrous or merely glandular-hispid.

## K. Infrastipular prickles absent.

- L. Flowering on laterals from two-year or older wood only. Flowering period short, definite. Upper stipules dilated.

## M. Stems unarmed, bristly only at base.

- N. Inflorescence 1-2. Petals 1.7-2 cm. Sepals reflexed and persistent on the fruit. Calci-phile plants. Bic, Quebec.....*R. williamsii*

2n = 14?

- NN. Inflorescence 1-20. Flowers subtended by ovate bracts. Sepals erect or spreading on fruit.

- O. Sepals 1.5-2.5 mm. wide, 8-23 mm. long, not narrowed toward base. Leaflets oval to obovate, serrations coarse, acute ascending, av. no. 12. Petals 1-2.5 cm. long. Stamens, av. no. 65. Fruit turning red in 3-4 weeks. Habitat west of 100th meridian.....*R. woodsii*

2n = 14

OO. Sepals 2-4 mm. wide.

P. Small-flowered slender roses found west of Sierra Nevadas. Leaflets ovate with cordate base and fine crenate teeth (av. no. 15). Sepals 2-3 mm. wide, 8-15 mm. long, constricted toward base. Stamens, av. no. 65. Petals 10-18 mm. Fruit needing over 2 months to ripen. . . . . *R. pisocarpa*  
2n = 14

PP. Large-flowered eastern roses. Leaflets oval or obovate. Teeth coarse, acute, ascending (av. no. 12). Petals 2-2.8 cm. Stamens 85-140

*R. blanda*  
2n = 14

MM. Stems bristly to tips or nearly so. Leaflets 5-7 (rarely 9). Habitat, Canada and northern U.S.A.

Q. Plants 0.3-2 m. tall. Early flowering.

R. Teeth ovate. Inflorescence 1-3 on laterals less than 7 cm. long. Petals 2.5-3 cm. Stamens 65-125. Early flowering northern plants. Fruits reddening in 3-4 weeks. . . . . *R. acicularis*  
2n = 42 and 56

RR. Teeth acute, ascending. Inflorescence 1-20 on laterals usually over 7 cm. long. Stamens 85-140. Flowers in June; fruits reddening in 5 weeks

*R. blanda* var. *hispida*  
2n = 14

QQ. Plants less than 3 dm. high, flowering after hexaploid and diploid species. Bristles short and weak. Flowers usually solitary. Habitat, northern Great Plains. . . . . *R. alcea*  
2n = 28



- LL. Flowering terminally in corymbs on suckers, as well as laterally on older wood. Everblooming. Flowering period extended.
- R. Stems unarmed, usually less than 3 dm. high.
- S. Leaflets 5, rarely 7, usually glandular. Hypanthium usually glandular-hispid. Pacific Coast forms. . . . . *R. spithamea*  
 $2n = 28?$
- SS. Leaflets 7-9, coarsely serrate. Flowers solitary or in small corymbs. Flowering on suckers rare. Habitat, northern Great Plains  
*R. alcea*  
 $2n = 28$
- RR. Stems bristly to tips.
- T. Leaflets 5, rarely 7, usually glandular. Hypanthium usually glandular-hispid. Semi-herbaceous dwarf. Habitat, Pacific Coast  
*R. spithamea*  
 $2n = 28?$
- TT. Leaflets 7-9 or 11, coarsely serrate. Teeth 7-21. Prairie forms; 0.1-1 m. high. Inflorescence 1-20, terminal or on laterals. . . . *R. arkansana*  
 $2n = 28$
- KK. Infrastipular prickles usually present.
- U. Stipules at least on the shoots, more or less convolute. Prickles curved. Leaflets pale beneath, eglandular  
*R. cinnamomea (introduced)*  
 $2n = 14$
- UU. Stipules flat.
- V. Inflorescence 1-3 on short laterals (3-10 cm. long).
- W. Stems bristly at base only, stout with more or less flattened prickles. Foliage often glandular and resin-scented.
- X. Stems over 1 m. tall, much branched, with straight ascending or recurved prickles. Petals 2.5-3 cm. Stamens 65-

115. Hip 10-18 mm. in diameter. Fruit firm, needing 10 weeks or more to ripen

*R. nutkana*

$2n = 42$

XX. Stems low, 0.2-1 m. Prickles often recurved. Petals 1.5-2.5 cm. Hip 1 cm. in diameter. Southern Rocky Mountain region. . . . . *R. manca*

$2n = 42$

WW. Stems bristly to top, slender or, if stout, simple and strict; height to 1 m.

Y. Hips usually erect, pedicels glabrous or glandular, 0.5-2 cm. long. Pacific Coast species.

Z. Stems strict, to 1.6 m., foliage often glandular.

a. Serrations fine, 9-17. Stem and pedicels often glandular-hispid. Prickles straight, flattened or terete. Leaflets usually densely puberulent and resin-scented. Petals 2 cm. long. Stamens about 70

*R. durandii*

$2n = 28$

aa. Serrations coarse, 4-14; leaflets 0.5-2.5 cm. Stems with scattered weak bristles and prickles. Petals 12-18 mm.

*R. californica* var. *bidenticulata*

$2n = 28$

ZZ. Stems low to 5 dm., with strict branches and numerous weak bristles and prickles. Leaflets to 2 cm. long, oval to orbicular. Petals 1.5-2 cm. Stamens 60-70. . . . . *R. yainacensis*

$2n = 28$

YY. Hips usually pendent. Pedicels slender, 1-3 cm. long.

- b. Early flowering. Petals 2.5 cm. Hips 1 cm. or more in diameter, reddening in 5-6 weeks. Stems low, with arching branches. Prickles and bristles slender. Rocky Mountain species

× *R. engelmannii*

$2n = 42$

- bb. Petals 10-15 mm. Hips less than 1 cm. in diameter. Stems slender, branched, prickles very weak. Sepals 4-5 mm. broad. Habitat, mountains of the far west.

- c. Sepals rarely caudate, about 10 mm. long, falling together with the disc. Pedicels often glandular-hispid. . . . . *R. gymnocarpa*

$2n = 14$

- cc. Sepals caudate, about 15 mm. long, erect or spreading and persistent on the ripe fruit. Dwarf of southern Sierra Nevadas. . . . . *R. calvaria*

$2n = 14$

VV. Inflorescence 1-15 or more. Flowering laterals usually over 10 cm.

- d. Sepals 1.5-2.5 mm. wide, not narrowed at base. Flowers on laterals from old wood. Buds tapering. Habitat, Great Plains and westward.

- e. Leaflets 5-7, 1-4 cm. long, serrations coarse and acute. Twigs with weak, rarely flattened infrastipular prickles. Stems 1-3 m. tall. . . . . *R. woodsii*

$2n = 14$

- ee. Leaflets 7-11, usually under 2.5 cm. long.

Twigs bristly or unarmed. Habit strict.  
Prairie form, usually less than 1 m. tall

*R. macounii*

$2n = 14$

dd. Sepals 2-4 mm. wide. Buds ovoid.

f. Small-flowered western roses with arching branches. Leaflets ovate, finely serrate, somewhat cordate at base. Flowers usually in corymbs. Buds ovoid.

g. Stems slender, sparingly armed. Serrations fine and crenate (av. no. 15). Petals 15 mm. long. Sepals narrowed at base. Flowers on laterals only

*R. pisocarpa*

$2n = 14$

gg. Stems coarse, often bristly and prickly. Petals 20 mm. long. Sepals slightly narrowed at base. Serrations 10-20 (av. 14). Flowering on long laterals and terminally on turions

*R. californica*

$2n = 28$

ff. Large-flowered roses of Great Lakes region. Coarse, strict habit with scattered, flattened prickles. Leaflets oval to obovate. Teeth ascending, 7-28 (av. 17). Petals 2-2.5 cm. Stamens 120-150

× *R. michiganensis*

$2n = 14$

# ORIGIN AND CELLULAR CHARACTER OF XYLEM RAYS IN GYMNOSPERMS

M. W. BANNAN

(WITH NINETEEN FIGURES)

## Introduction

Although much work has been done on the structure of the xylem ray in gymnosperms, its origin and cellular character apparently have not been investigated from a comprehensive comparative viewpoint. In the present work it is proposed to trace the ray through from its origin, to note the distribution and character of the cells in the different parts of its course, to determine how this distribution and character vary with the location of the ray in the tree (whether in root or stem), and to establish the nature of the changes from the inner to the outer wood. Since *Pinus* affords the greatest variety of cell types, the rays in this form will be described in some detail; the condition in other gymnosperms is considered more briefly.

In the description of rays, the term central, instead of the term interspersed, is applied to the rows in the interior of a ray, the latter term being reserved to describe the alternation in arrangement of different types of cells, in the same radial row, whether that row be marginal or central. Ray cells of the same row, when separated, are referred to as scattered; when in radial contact, as contiguous. Hitherto the term interspersed has also been used to describe the scattered cells of marginal rows.

In the illustrations the pith or primary wood is always toward the left, and the limits of the annual rings are marked by vertical lines. In some of the figures the wall characters are shown; in other figures the ray tracheids and tracheids are represented simply by outlines, "ghosts" by dotted lines, and parenchyma cells by stippling. The stippling merely indicates that the cells are parenchymatous, and does not represent cell content, for in some cases the cells are empty.

### Ray origin and cellular character

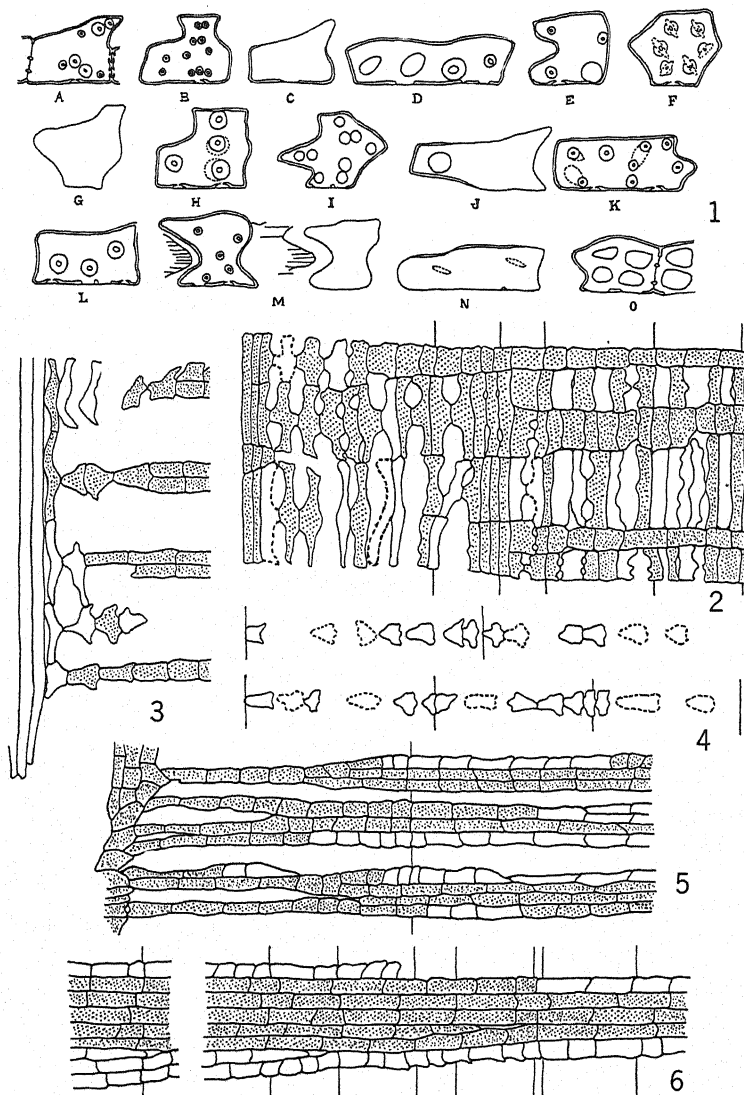
#### PINUS

In the roots examined, taken from different depths of soil and at different distances from the stem, a considerable amount of variation was observed. It was found that in roots near the stem, or in roots which had been exposed on banks or escarpments, the wood was similar to that in the stem and unlike that in buried roots. Owing to these dissimilarities the two types of roots are considered separately.

In a buried root of a soft pine such as *P. strobus* the rays originating at primary tissue consist of vertically elongated cells, which are either in radial contact or only slightly scattered. Several ray rows are usually found in a vertical series, the earliest cells of each row being in contact with the cells above and below. In some cases the succeeding cells become shorter and the rows separate into individual rays; in other cases there is no shortening and a high ray results. The cells are parenchymatous, although in the succeeding parts an occasional ray tracheid may be interspersed.

Rays arising in the secondary wood are of similar form to those described, but each series of rays succeeds a row of tracheids, with the upper and lower rays of the series in line with the ends of the tracheids, indicating that the ray initials were formed by segmentation of fusiform initials. Although it is usual for the whole fusiform initial to be concerned in the formation of ray initials, occasionally only the upper or lower part is segmented and but one or two ray initials produced. As in the case of the rays beginning at the center, the earliest cells are vertically elongated and are radially contiguous or slightly scattered (fig. 2). Where the succeeding cells become shorter the rows separate into rays, but when such shortening does not occur the rows form a high ray. Most of the rows sooner or later are followed by two or more, the ray initial being horizontally divided.

In the rays originating in the secondary wood, especially in those whose origin is some distance from the center, many different types of cell are found (fig. 1). The earliest cells of the rays are usually tracheary or ghost-like. The ray tracheids as a rule have bordered



FIGS. 1-6.—Figs. 1-4, *Pinus strobus*: Fig. 1, types of ray cell in root wood (dotted lines represent outlines of pores in walls of adjoining tracheids). Fig. 2, portion of ray in root wood. Fig. 3, origin of rays in young stem wood, 0.5 mm. from pith. Fig. 4, new ray in old stem wood 11.5 cm. from pith. The ray is traced through five annual rings. Fig. 5, *P. banksiana*, ray tracheids succeeding parenchyma cells in rays which originated at the pith. Fig. 6, *P. resinosa*, ray in old stem wood 15 cm. from pith showing dying of tracheary rows and development of ray tracheids in parenchymatous row.

pits in all walls touching other cells, but there is considerable variation in the size of the pits, as indicated in types *B*, *H*, and *L*. Other cells may have both tracheary and parenchymatous characters, such as bordered and simple pitting, with the simple pits on radial walls (*D*, *E*), horizontal walls (*D*, *L*), or tangential walls (*A*). There may also be irregularities in the pits of the ray cells and the adjoining tracheids, both in size and character of the pits. For instance, simple or but slightly bordered pits in the tracheids may overlie bordered pits in the ray cells (*H*, *K*). Interspersed among the tracheary cells are many ghost-like types. These show gradations from faint outlines (*G*) on the one extreme to parenchyma cells with small pits (*I*) opposite bordered pits in the neighboring tracheids, on the other extreme to parenchyma cells with large pits (*O*) opposite simple pits in the tracheids. In the intermediate types the walls are thickened and may be unpitted (*C*, *N*) or pitted (*J*). The ghost-like cells, ray tracheids, and many of the parenchyma cells lose their protoplasm shortly after differentiation. The wall character of the ordinary parenchyma cell is shown in (*O*).

In addition to the ghosts, intercellular tracings or markings (fig. 1, *M*) often occur near the origins of rays, where the cells are vertically rather than radially extended. These are probably indicative of a displacement of ray cells in relation to tracheids, resulting from different rates of division of ray and fusiform initials and a failure of the ray cells to extend radially.

The rays beginning at the center of the root are usually entirely parenchymatous throughout their course, although in a few cases ray tracheids may be interspersed among the parenchyma cells in that part of the ray some distance from the center. On the other hand, the rays originating in the outer secondary wood are at first composed of tracheids and ghost-like cells with parenchymatous types interspersed. In the succeeding parts of these rays the parenchyma cells become more numerous and eventually the rays are composed wholly of this type of cell. Outward from the center there is an increasing development of ray tracheids, principally in the first formed parts of the successive new rays, but also to a lesser degree in the parenchymatous rays of early origin.

In exposed roots the rays resemble those in the stem, and as the



latter will be described in some detail, the rays in this type of root will not be further considered. Between stemlike roots on the one hand and buried roots on the other there are many intermediate gradations.

Rays arising at the pith of the stem of *P. strobus* consist of contiguous parenchyma cells. As in the root, several ray rows are usually found in a vertical series, the earliest cells of each row being vertically elongated and in contact with the cells above and below. The succeeding cells shorten more rapidly than in the root, however, and the rows soon separate into rays.

The rays originating in the innermost secondary wood are of like character, but at a distance of 0.3 mm. or more from the pith a change is noted. Ray tracheids appear in some new rays and occasionally also in rays which began at the pith. When in new rays they may be either the first cells or be preceded by one or two ray parenchyma cells (fig. 3), but in any case there are few ray tracheids in a single ray. A slight scattering of the first cells of the rays may also be observed.

In the outer, as in the inner wood, the new rays as a rule are found in a vertical series; but here, in contrast to the condition in the inner wood, there are considerable gaps between the last tracheids and the first cells of the succeeding new rays. The earliest ray cells are scattered and there is little or no transition from vertically to radially elongated elements, even the first cells being short and somewhat radially extended (fig. 4). The new rays are usually only a single cell high, and the cells are for the most part tracheary, although ghosts may be interspersed. In the succeeding parts of the ray the cells are longer, and also closer, so that they come into contact. The initial of the ray, which may yet be a single cell high, is then horizontally divided, sometimes successively, and the number of rows increased. While the ray is increasing in height the tracheary character continues in the marginal rows, and the central rows on the other hand become parenchymatous. In the outer wood from 30 to 50 per cent of the total ray tissue is tracheary.

In the stem the newly formed ray initials shorten more rapidly than in the root. The ray initials formed by the segmentation of a single fusiform initial soon separate, producing a number of separate

rays one cell in height rather than one or two rays several cells high, such as often occurs in the root. The older rays in both root and stem add to their height by doubling some of the rows, but this doubling occurs more often in the root. Consequently, owing to the greater initial height and the faster rate of increase, the rays in the root are much higher than in the stem.

Outward from the center in both root and stem there is an increasing development of ray tracheids, mostly in the earliest parts of the new rays, but also in the succeeding parts of the formerly parenchymatous rays which originated at the center. There are, however, considerable differences in the numbers of the various types of cell between root and stem. In the stem there are many more typical ray tracheids, and in proportion to these, fewer poorly developed ray tracheids, fewer ghost-like cells, and fewer cells intermediate in character between ray tracheids and parenchyma than in the root.

Compared with the soft pines, in the hard species ray tracheids appear earlier, are more abundant in old wood, and are of more elaborate form. Unlike the soft pines, ghost-like cells are scarce in the root, and are rarely found in the stem. There are likewise fewer cells with bordered and simple pitting, etc. Practically all the cells are either typically tracheary or parenchymatous, the former constituting 50 per cent or more of the ray tissue in old stem wood.

The sequence of the two principal types of ray cell, tracheary and parenchymatous, has been a subject of controversy among anatomists. In the pines KNY (6) illustrated the formation of ray tracheids, either as new rows or as succeeding parenchyma in older rows. SCHMIDT (9) and THOMPSON (10) described the origin of new tracheary rays, THOMPSON stating that ray tracheids were ultimately followed by parenchyma. PENHALLOW's theory (7) on the origin of ray tracheids from parenchymatous tissue was interpreted by THOMPSON as necessitating an actual sequence of parenchymatous cells followed by ray tracheids, but he did not find such an arrangement.

As noted in the foregoing description, the rays originating in the secondary wood are at first tracheary, or predominantly tracheary (parenchyma cells may sometimes be interspersed among the ray tracheids), and later become parenchymatous. The reverse order,

that of parenchyma followed by ray tracheids, occurs in addition however. In young stem wood many of the marginal parenchymatous rows of rays originating near or at primary tissue are succeeded by tracheary rows (fig. 5), this sometimes occurring to such an extent in the hard pines that even close to the pith half the ray rows become tracheary. Many of the tracheary rows which succeed parenchyma are in turn followed by parenchyma in old wood.

The sequence of parenchymatous followed by tracheary cells may also be seen in old stem wood. Such change frequently occurs in submarginal rows of old rays, in which case it is usually correlated with the cessation of the adjoining marginal row (fig. 6). The upper marginal tracheary row has ceased, and the submarginal parenchymatous row has become tracheary after assuming a marginal position. In some cases a row such as the latter in turn dies and the parenchymatous row within becomes a marginal tracheary one. Sometimes the submarginal row attains tracheary characters before the marginal row ceases, and instances were observed where the change took place in the submarginal row although the marginal one continued unaltered. The sequence from parenchymatous to tracheary cells occurs less frequently in the central rows. As in the marginal rows, the change is sometimes associated with the dying of the row; that is, when a parenchymatous row ceases some of the last cells may be tracheary instead of parenchymatous. In other cases a few ray tracheids are simply interspersed in otherwise normal parenchymatous rows.

Although the dying of tracheary ray rows is of common occurrence in old wood, it occurs more often in the slowly growing parts. This relation was shown most clearly in an old tree of *Pinus resinosa* which was growing in a burnt-over area and had a serious fire wound at the base of the stem. The growth rings preceding the year of the fire were wide but afterwards were very narrow. In a given area in the growth ring preceding that of the wounded year, but some distance above the wound, there was a net gain of ten ray rows whereas in the corresponding area in the five narrow years there was a net loss of forty-two rows. In the six years fifty-five rows ceased, of which fifty-four were tracheary. Owing to the fact that some parenchymatous rows became tracheary, however, the percentage reduc-

tion of tracheary tissue was relatively slight, from 51 to 46 per cent. Similar relations between the ring width and the character of the ray tissue were noted in slowly growing but unwounded trees.

The last ray tracheids in the dying rows are of various shapes. Sometimes they are long and radially attenuated; in other cases they are shorter than usual. The latter condition is illustrated in the upper row in fig. 6. After the last ray tracheid there are as a rule no ghosts. Exceptions to this condition are rare, and are for the most part limited to such locations as the dying rows of recently formed rays in soft pines, where ghosts occur in any case. The lack of ghosts in dying tracheary rows is of special significance in the interpretation of ghost-like cells.

#### OTHER GYMNOSPERMS

In the case of the other gymnosperms studied, the rays in the inner stem wood are composed of contiguous parenchyma cells, with the earliest cells of each ray showing gradations from vertically to radially elongated elements, such as described in *Pinus*. From the rays of this character, found in the inner wood, there is a sequence to that type of ray peculiar to the species in old wood.

In the roots the transitional stages from the first high cells of the new rays to the succeeding radially extended ones last longer than in the stem. The rays are higher, and even in old roots are more largely parenchymatous than in stems of like size or age. Sometimes the differences in cellular character of the rays in root and stem are very great. For instance, in *Picea*, where ray tracheids constitute 30 per cent of the ray tissue in mature stem wood, old roots of the buried type were found which were entirely lacking in ray tracheids, and in others these cells occurred only sporadically.

Since the rays in the innermost stem wood and in the root of these gymnosperms are of more or less uniform origin and cellular character they will not be further considered. There is, however, much variation in the shape, arrangement, and character of the ray cells in old stem wood. Consequently the condition in the old stem will be more fully dealt with, the forms with parenchymatous rays being described first.

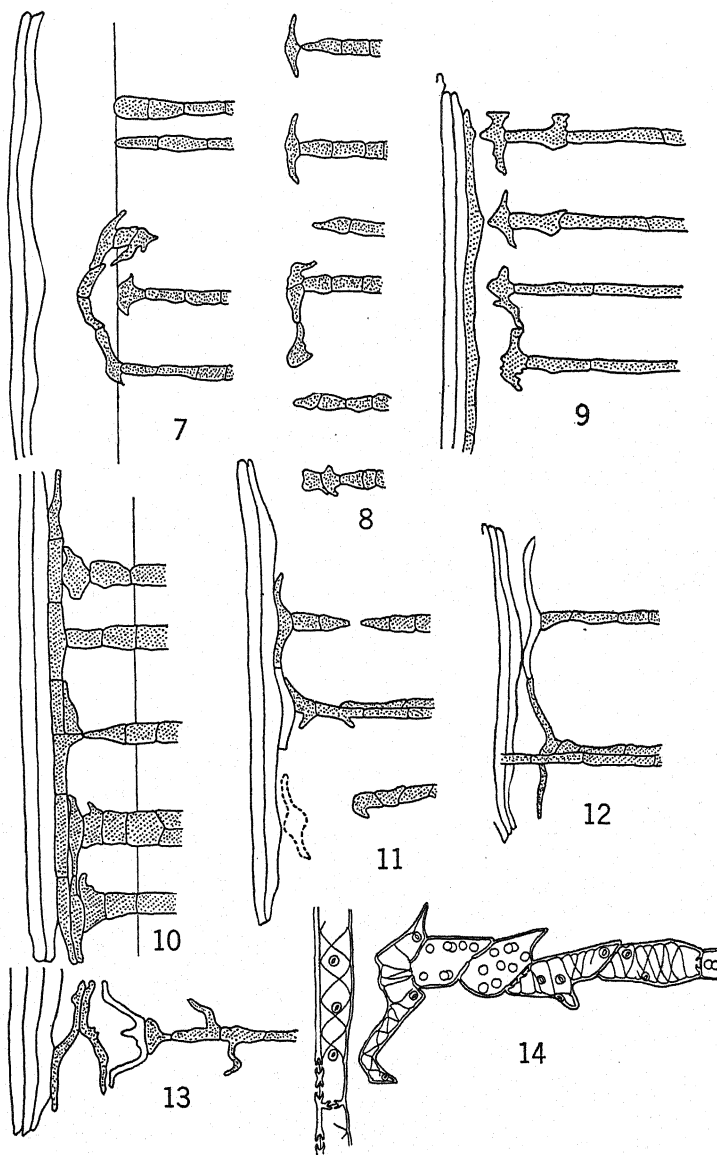
In the Cycadales *Dioon spinulosum* was selected, because of the

width of the zone of wood. The new rays succeed radial rows of tracheids or xylem parenchyma, with the first cells of the rays in contact with or close to the last fusiform elements. All the ray cells are contiguous and parenchymatous.

The new rays in old wood of *Ginkgo biloba* succeed tracheids, septate tracheids, or xylem parenchyma, with or without short gaps between the rays and the fusiform cells (fig. 7). The first ray cells are sometimes vertically extended, but more often are short and somewhat elongated radially, the transitions from vertically to radially elongated cells being largely omitted. The earliest cells of the rays are usually parenchymatous and thin-walled, but in rare instances are thick-walled, and in very exceptional cases may be tracheary. The succeeding cells are contiguous and always parenchymatous.

Rays in stem wood of the Araucarineae (fig. 8) are similar to those in the stem of *Ginkgo*, except that the gap between the last tracheids and the first ray cells may be slightly greater, the earliest cells are more often radially extended, and typical ray tracheids occur though only sporadically. The rays are usually composed entirely of thin-walled parenchyma, but in rare instances a few of the earliest cells are tracheary. These cells were found only near the ray origins, a distribution which is different from that noted by POOL (8), who described them in the central rows of old rays. Ghosts may be observed in an occasional new ray but their numbers are exceedingly small. Thick-walled parenchyma cells are sometimes present, both in new and old rays.

In the Podocarpineae, *Dacrydium*, *Microcachrys*, *Phyllocladus*, *Saxegothaea*, and *Podocarpus* were studied. The origin of rays in the stem is illustrated in figure 9. The new rays succeed tracheids or xylem parenchyma, with little or no gap between, and consist of contiguous parenchyma cells, the earliest of which show transitions from vertically to radially elongated shape. The only species in which ray tracheids were found was *Microcachrys tetragona*. Here they were of rare occurrence, having been found only in new rays, with never more than one tracheid in a single ray. The tracheary containing rays were sparsely distributed throughout the wood of the stem and showed no correlation with injury. The ray parenchyma cells in the Podocarpineae have different degrees of wall thickening



FIGS. 7-14.—Newly formed rays in stem wood: Fig. 7, *Ginkgo biloba*. Fig. 8, *Agathis alba*. Fig. 9, *Podocarpus elatus*. Fig. 10, *Torreya nucifera*. Fig. 11, *Taxus baccata*. Fig. 12, *T. brevifolia*. Fig. 13, *T. baccata*. Fig. 14, *T. canadensis*, showing tracheary cells in ray.

and lignification, and there is considerable variety in the pitting between ray cells and tracheids.

The rays of such Taxineae as *Cephalotaxus* and *Torreya* exhibit features similar to those of the Podocarpineae. The earliest cells of the rays are usually vertically elongated and the following ones lower (fig. 10). The ray cells are almost invariably contiguous and are parenchymatous.

In *Taxus* the rays succeed tracheids with little or no gap intervening. The first cell of the ray is frequently very high, and the following ones much shorter, the transition from high to low cells being quickly passed over. In the majority of the rays all the cells are parenchymatous, but in some cases one of the earlier cells is tracheary, as in figures 12 and 13, and in a few cases typical ray tracheids are present (fig. 14). The ray tracheids are always near the ray origins but are not necessarily the first ray cells, there being no fixed sequence of ray tracheids and parenchyma. Not more than three ray tracheids were found in a single ray, and the rays with such cells were few in number, occurring sporadically throughout the stem. The walls of the tracheary ray cells show spiral thickenings and as a rule have few pits, the tangential walls sometimes lacking them entirely. Ghosts, of which one is shown in the lower ray in figure 11, are of rare occurrence.

It has been mentioned that the earliest cells of most of the new rays are in contact with or only slightly removed from the preceding fusiform elements, and that the ray cells themselves are contiguous. This arrangement is significant for it indicates that there can be no question of ray tracheids having been once present but lost by dropping out.

In the different Cupressineae studied a considerable amount of variation was found in the rays of old stem wood. Here, as in old wood generally, the new rays are usually one cell high. In some cases the majority of the cells of the new rays are parenchymatous, with a few ray tracheids interspersed, and in others the rays are largely tracheary, with parenchyma interspersed. The earliest cells are radially extended and scattered, with gaps between them and the last of the preceding tracheids. The gap is usually widest and the scattering greatest in the rays where the tracheids are the most nu-

merous. When the ray is traced outward in the older wood the parenchyma cells interspersed among the tracheids increase until the ray becomes entirely parenchymatous, and at this point the cells are usually contiguous. The initial of the ray, which as a rule is still a single cell high, may then be horizontally divided, producing a parenchymatous ray two cells or more in height. Where a marginal row of an old ray is tracheary this is due to the addition of an entirely new ray to the margin, rather than to a continuance of the tracheary character in the ray itself.

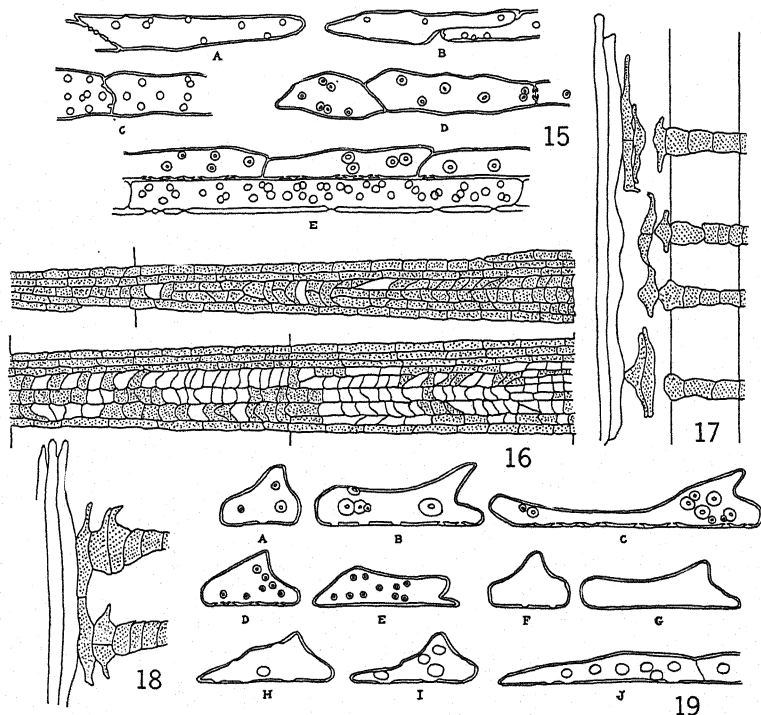
Ray tracheids were abundant in the material of some of the species studied and rare in others; but since similar variation was observed in those forms where a choice of material was available, such as *Thuja* and *Juniperus*, it is evident that the differences cannot be considered necessarily specific or even generic. Ray tracheids were found in varying number in stem wood of all the genera studied (*Callitris*, *Chamaecyparis*, *Cupressus*, *Juniperus*, *Libocedrus*, *Thuja*, *Widdringtonia*), and the greatest number noted was in an old stem of *Thuja occidentalis* where 6 per cent of the ray rows contained tracheary cells.

Some of the different types of cells found in new rays are illustrated in figure 15 from *Thuja occidentalis*. The ray tracheids have large, or small, or both large and small bordered pits on radial walls but frequently lack them on tangential walls (*D*, *E*). The majority of the parenchyma cells have thin tangential walls (*B*, *E*) but a few have thickened ones (*A*, *C*). Their radial walls are usually abundantly pitted (*C*, *E*) but are occasionally only sparingly so (*A*, *B*). Some of the parenchyma cells, particularly those with few pits such as the *A* and *B* types, are characterized by the early disappearance of their protoplasm. Empty thin-walled unpitted cells and ghosts were not observed. The older rays are composed of ordinary parenchyma cells with thin tangential walls, abundantly pitted radial walls, and intact protoplasm (*E*).

In addition to the rays described, others of a peculiar type were occasionally found in *Thuja occidentalis*. One of these is illustrated in figure 16. The abnormal feature is the development of ray tracheids in the central rows of the ray, which in this particular case is all the more outstanding since the ray originated at primary tissue. Where



ray tracheids succeed parenchyma in the pines, it is usually either in marginal rows or in dying central rows; but neither of these conditions holds in *Thuja*. The abnormal rays were found in both young and old stem wood, but considering the large number of specimens examined are rather rare. No correlation with wounding was evi-



FIGS. 15-19. Figs. 15-17, *Thuja occidentalis*: Fig. 15, types of ray cell in old stem wood. Fig. 16, abnormal ray with ray tracheids in central rows. Fig. 17, newly formed rays in root wood, illustrating character of rays in root and young stem wood. Fig. 18, *Cunninghamia lanceolata*, character of new rays in young stem wood. Fig. 19, *Sequoia sempervirens*, types of ray cell in old stem wood.

dent. Rays of like character were not observed in other species, although HOLDEN (4) has described somewhat similar rays from *Sequoia gigantea* and POOL (8) from *Araucaria*.

In the Taxodineae the differences in ray structure are apparently recognizable as generic. Based upon the material examined, which in some cases was rather limited, the genera are divisible into three

groups: *Athrotaxis* comprises one, *Taxodium* and *Cryptomeria* the second, and *Sequoia*, *Cunninghamia*, and *Taiwania* the third.

New rays throughout stem wood of *Athrotaxis* almost invariably consist of contiguous or only slightly scattered parenchyma cells, with the earliest cells close to or in contact with the preceding tracheids. Ghosts and thin-walled sparsely pitted empty cells are apparently absent, and ray tracheids very rare.

In *Taxodium* and *Cryptomeria* the new rays consist of scattered cells which are either ghost-like or have thin, sparsely pitted walls and are devoid of content. Ray tracheids occur only rarely, interspersed among the ghosts or empty cells. In the succeeding parts of the rays, thicker walled empty cells, resembling types *F* to *J* in figure 19, appear, and so increase as to constitute the bulk of the tissue. These cells in turn are followed by contiguous parenchyma cells of ordinary character. The ghosts, empty cells, and occasional ray tracheids comprise from 5 to 10 per cent of the ray tissue in old wood.

*Sequoia*, *Cunninghamia*, and *Taiwania* differ from *Taxodium* in having fewer ghosts and more abundant ray tracheids. If a ray arising in old wood is followed through from its origin, it is found that the earliest cells are scattered and mostly tracheary; but outward there is an increase in interspersed parenchyma and cells combining tracheary and parenchymatous characters, accompanied by diminishing spaces between the cells, until the ray is entirely made up of contiguous parenchyma. The old rays are several cells high and are usually wholly parenchymatous, although sometimes ray tracheids may be observed in the marginal rows. In most cases, however, the latter condition is due, not to a continuation of the tracheary character through from the origin of the ray itself, as in the pines, but to the addition of entirely new rays (which are tracheary like all new rays in old wood) to the margin of the older ray.

The different types of ray cells in these forms are illustrated in figure 19 from *Sequoia sempervirens*. The majority of the ray tracheids have bordered pits in all walls touching other cells (*C*), but in some cases lack them on tangential or horizontal walls (*E*). The pits vary considerably in size (*B*, *C*). Other cells, such as described by GORDON (3), have both bordered and simple pits (*A*, *B*), with the

simple pits on horizontal walls adjacent to ray parenchyma. Ghosts are rare, and cells with slightly thickened and lignified but unpitted walls are of occasional occurrence. From the latter one may observe gradations in cells with thickened but sparsely pitted walls (*F*, *H*, *I*), the majority of these being without content. Although all these types may be recognized in new rays, most of the cells are either typical ray tracheids or parenchyma. Approximately 10 per cent of the ray rows in old wood of *Sequoia sempervirens*, and 5 per cent in *S. gigantea*, *Cunninghamia*, and *Taiwania* are largely composed of tracheary cells, the remainder of the rows being wholly parenchymatous.

Old wood of *Sciadopitys* was not available, but in young stem and root wood the rays were entirely parenchymatous, as in the similar regions of the other Taxodineae (fig. 18).

The cells in the newly formed rays in old wood of some Taxodineae are widely scattered, more so than in other conifers. Where such scattering is found, outlines of cells never occur in sufficient numbers to make the rows contiguous. Occasionally the separated ray cells have ghost-like pithward-extending appendages, which obviously are not vestiges of separate cells but are evidence that the ray cells and tracheids have moved in relation to each other. Sometimes the tracheid lying side by side with and just preceding a ray cell in the scattered row has small pits on that part of its wall in line with the ray cell. Such phenomena indicate that the extent of the scattering is dependent upon the rate of division of the ray initials, rather than upon the breakdown and disappearance of cells. The spaces cannot be interpreted as the result of a loss of ray tracheids, which cells some investigators consider have largely disappeared from the Taxodineae.

The distribution of ray tracheids in the Taxodineae and Cupressineae has been dealt with by several investigators, but most extensively by HOLDEN (4). She has described ray tracheids in several species as being more abundant in wounded material, and considered these cells traumatic. JEFFREY (5) likewise believes ray tracheids to be traumatic reversions to an ancestral condition. The writer, however, was unable to find evidence in support of such interpretation. Wounded specimens of several genera (*Callitris*, *Cupressus*, *Chamae-*

*cyparis*, *Juniperus*, *Thuja*, *Sequoia*, *Taxodium*, and *Cunninghamia*) were examined, but an increase in ray tracheids, either in the parts adjacent to or opposite the wounds, was not found when comparison was made with normal wood.

In the sub-tribe Abietae of the Abietineae generic differences in ray structure are further accentuated. Rays in mature stem wood of *Keteleeria* contain numerous ghosts but no ray tracheids. *Abies* is similar except that ray tracheids are present, although rare. *Cedrus* has fewer ghosts and more ray tracheids, and in *Tsuga* there are exceedingly few ghosts and abundant ray tracheids.

The cells of the newly formed rays in *Keteleeria* are scattered and of the following types: ghosts, empty cells with slightly thickened but unpitted walls, gradations from these to parenchyma with typical wall structure but devoid of content, and finally ordinary parenchyma. The ghosts are most numerous toward the ray origin, and the other types become more abundant in the succeeding parts. In old rays the ghost-like character may continue in the marginal rows, the ghosts occurring in the spring and parenchyma cells in the summer wood of each annual ring, while the central rows are exclusively parenchymatous. From 20 to 25 per cent of the ray rows in old wood contain ghosts, the remainder being parenchymatous.

In the case of *Pseudolarix* old stem wood was not studied, but in young material the rays resembled those in *Keteleeria* of like age.

The rays of *Abies* are similar to those of *Keteleeria*, except that the changes in ray character outward from the pith are of more rapid sequence and that ray tracheids are of sporadic occurrence. Cells with both bordered and simple pits, as described by THOMPSON (11) from *Abies homolepis*, occur rarely. The ghosts, thin-walled empty cells, ray tracheids, etc., constitute approximately 25 per cent of the ray tissue in old wood.

The erratic nature of the distribution of ray tracheids in *Abies* is evident from the work of several investigators. PENHALLOW (7), DEBARY (2), and WIESEHUEGEL (12) noted ray tracheids in *A. balsamea*, but HOLDEN (4) and THOMPSON (11) failed to find any in this species. THOMPSON described these cells in *A. amabilis*, *A. concolor*, *A. homolepis*, and *A. veitchii*, however, and considered them traumatic. Extensive material of *A. balsamea* was examined by the

writer and ray tracheids were found sparingly in a few specimens but were absent from the majority. Although sometimes occurring in wounded areas, the ray tracheids were absent from many other wounded specimens, and present in others which showed no evidence of injury. The distribution could not be correlated with wounding or vigor of growth. Ray tracheids were observed also in *A. concolor*, *A. homolepis*, *A. lasiocarpa*, and *A. veitchii*. Here again they were not necessarily connected with injury. In fact, in a wounded specimen of *A. concolor*, a species in which THOMPSON described traumatic ray tracheids, these cells were actually less numerous in the wounded year's growth than in the preceding unwounded wood.

The cells in the rays of *Cedrus* are of similar types to those in *Abies*, but ghosts are less numerous, the majority of the cells in the first formed parts of the ray being tracheary. The ray tracheids, ghosts, thin-walled empty cells, and interspersed parenchyma of various types comprise from 12 to 25 per cent of the ray tissue in old wood.

In contrast to other Abietae, ghosts are rare in *Tsuga canadensis*. The earlier cells of the rays are usually somewhat scattered and are tracheary almost without exception. With subsequent increase in height of the ray, the tracheary character persists in the marginal rows; although in this part of the ray parenchyma cells are often interspersed among the ray tracheids, especially in summer wood. Approximately 30 per cent of the ray rows in old wood are tracheary.

*Tsuga caroliniana* resembles *T. canadensis*, but in *T. heterophylla* and *T. mertensiana* the first ray tracheids appear farther outward from the pith; and in old wood these cells constitute 15 to 25 per cent of the ray tissue, about 10 per cent less than in *T. canadensis*.

In the sub-tribe Pineae, *Larix*, *Picea*, and *Pseudotsuga* have similar rays. As in *Tsuga*, the cells of the new rays are somewhat scattered, with the first cells some distance from the preceding tracheids. The new rays are a single cell high and are exclusively tracheary. In the succeeding parts, where the ray is higher, the tracheary character present in the earlier part of the ray continues in the marginal rows, and the central rows become parenchymatous. In some cases, as noted in the Cupressineae and Taxodineae, new tracheary rays may be added to the margins of old rays, but in *Picea*, *Larix*, *Pseudotsuga*, and also in *Tsuga* the majority of the tracheary marginal rows may

be traced back to the origin of the ray itself. Unlike the condition in *Tsuga*, parenchyma cells are rarely interspersed among the ray tracheids, unless as vertical resin canals. Practically all the cells are either typical ray tracheids or parenchyma; ghosts, thin-walled empty cells, etc., are rarely found. Usually 30 per cent, but at times as much as 50 per cent of the ray rows are tracheary.

In the rays arising in the old wood, the earliest parts are tracheary and the succeeding parts (in the central rows) parenchymatous. The sequence of cell types for the central rows is that of ray tracheids followed by parenchyma. The reversed order, that of parenchyma cells succeeded by ray tracheids, may also be observed in *Larix*, *Pseudotsuga*, *Picea*, and to a lesser degree in *Tsuga*, under such circumstances as have been described in the pines. In all the other conifers ray tracheids occur almost invariably only in new rays and, except for the parenchyma cells interspersed among ray tracheids, very rarely succeed parenchyma.

### Discussion

In the description of rays evidence has been given for the formation of ray initials by segmentation of fusiform initials. In the behavior of the ray initials, however, and also in the character of the daughter ray cells, there is considerable variation. The differences will be further considered and their significance discussed.

In stem wood of a gymnosperm such as *Dioon*, the ray initials formed by the segmentation of a single fusiform initial lie in a vertical series, each of the initials touching those above and below. The ray initials continue dividing at approximately the same rate as the neighboring fusiform initials, and in consequence the earliest cells of the rays are high and narrow and in contact with similar cells above and below. Subsequently the initials shorten and separate. They divide more slowly, and in the succeeding parts of the rays, which have now separated, there are graded series from the first vertically elongated cells to those of lower and somewhat radially extended shape. All the cells produced by the ray initials are parenchymatous. This type of ray is found in *Dioon* and, with slight modification throughout stem and root wood of the Taxineae and Podocarpaceae, and in the root and innermost stem wood of most conifers.

The second type of ray is characterized by the appearance of gaps

between the last fusiform elements and the first ray cells, and by the omission of some or all of the transitions from vertically to radially elongated cells. In some cases the earliest cells of the rays are radially separated, this being the result of slow ray initial division. All the ray cells are parenchymatous. Such rays are found in mature stem wood of *Ginkgo*, the Araucarineae, and some of the Cupressineae and Taxodineae.

In the third type the general form of the ray is similar to that just described, but some of the earlier cells are tracheary instead of parenchymatous. Rays of this type occur sporadically throughout mature stem wood of certain Araucarineae, Podocarpineae, and Taxineae, in greater numbers in Cupressineae and Taxodineae, and most abundantly in young stem wood of the majority of the Abietineae.

The changes in the character of the ray origin, noted in the second and third types of rays, are accentuated in the fourth type. The gaps between the first ray cells and the preceding tracheids are often of considerable extent, and the transitional stages from high to low and radially extended cells are usually entirely omitted. The majority of the earlier cells of the rays are tracheary. With the increased development of ray tracheids these cells, which at first are found only in the earliest parts of the rays, extend into the succeeding parts and, at the expense of the parenchyma cells, constitute the marginal rows of the older rays. Rays of this character may occur sporadically in old stem wood of such Taxodineae as *Sequoia*, and are characteristic of the mature wood of most of the Abietineae.

Ultimately ray tracheids appear also in ray rows which were formerly parenchymatous, both in rays originating in the secondary wood and in rays arising at the pith. Such development is best seen in the stem of the hard pines, but also occurs in *Picea*, *Larix*, *Pseudotsuga*, and to a limited extent in *Tsuga*. The appearance of ray tracheids in parenchymatous rows beginning at the pith is of particular significance, for it indicates that the tracheary character may be attained by all ray cells regardless of the point of ray origin.

There is then a sequence from parenchymatous rays, composed of contiguous cells, in such admittedly primitive gymnosperms as the cycads, to tracheary rays with scattered cells (lacking the transitions from vertically to radially elongated cells) in such Abietineae

as the pines. This sequence in its general features parallels that outward from the inner to the outer wood in pines and other conifers with ray tracheids in mature wood. Further, in the fossil forms, except those of Abietinean affinity, there is a general absence of ray tracheids. These parallels in sequence from parenchymatous to tracheary rays therefore indicate that ray tracheids are a newer type of cell appearing in the ray in place of parenchyma.

The view that ray tracheids replace parenchyma, and that a sparing occurrence of the former is indicative of initial stages of acquirement, was advanced by PENHALLOW (7). On the other hand JEFFREY (5), HOLDEN (4), THOMPSON (11), and CHRYSLER (1) have presented evidence which they consider indicative of a loss of ray tracheids. Their conclusions were based upon such features as the occurrence of ray tracheids in or near wounded areas, and on the interpretation of ghosts and parenchyma cells interspersed among ray tracheids. The bases of such interpretation will be more fully considered.

The writer examined wounded specimens of several genera but an increase in the number of ray tracheids was not observed when comparison was made with normal wood. The locations in which traumatic ray tracheids have been described, such as the wound cap and the side opposite the wound, were studied in particular, but ray tracheids were not found to be more abundant there than in other parts of the tree. Evidently ray tracheids are to be considered characteristic of normal wood, rather than traumatic reversions.

In regard to the ghosts described by THOMPSON in *Abies* and considered by him to represent vestigial ray tracheids it is significant that these are found abundantly in not more than six or seven genera of the conifers, and if such a loss of ray tracheids has taken place it has been only in these few genera, belonging to the Abietae and Taxodineae. In all the other gymnosperms the rays are composed either of parenchyma cells, or of parenchyma and ray tracheids and their various intergrades, typical ghosts being scarce or absent. Hence if these forms (Araucarineae, Podocarpineae, Taxineae, Cupressineae, and most Taxodineae) have lost ray tracheids, such cells must have been replaced by parenchyma. On the contrary, the evidence of phylogeny and of the ontogenetic sequence indicates that the replacement has been one of parenchyma by tracheary cells.



From a theoretical standpoint it is also of interest to note that in such regions as the female cone axis and the root, which have been regarded by a number of investigators as conservative, ray tracheids are absent or less numerous than in the stem.

### Summary

1. The origin and cellular character of rays originating at different points in the xylem of stem and root were studied from representative gymnosperms.

2. The ray initials evidently are formed by segmentation of fusiform initials.

3. The newly formed rays in *Dioon* are wholly parenchymatous, as are also the majority in *Ginkgo*, Araucarineae, Podocarpineae, and Taxineae. In the stem of the latter groups ray tracheids occur sporadically in occasional new rays.

4. Rays in the inner wood of the Cupressineae, Taxodineae, and Abietineae are parenchymatous, but in old stem wood ray tracheids occur in varying degrees of abundance. In the Cupressineae and Taxodineae the ray tracheids as a rule are found only in new rays, but in the Abietineae they appear also in rays which originated earlier and which were formerly parenchymatous.

5. Roots are variable in structure. In buried roots the rays are more largely parenchymatous and less tracheary than in the stem, while in exposed roots the rays resemble those in the stem.

6. The sequence in ray origin and cellular character outward from the pith in living forms, the condition in the different groups of gymnosperms, and the fossil evidence, all indicate that the primitive ray is parenchymatous and that ray tracheids have arisen at the expense of parenchyma.

I wish to express gratitude to Professor R. B. THOMSON, both for his kindly criticism and assistance and also for the material made available by him. For supplementary material I am indebted to Professor J. H. WHITE of the Faculty of Forestry.

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# STUDIES ON THE GERMINATION OF THE SPORES OF CERTAIN BASIDIOMYCETAE

FRED H. O. KAUFMANN

(WITH TWO FIGURES)

## Introduction

This investigation was undertaken for the purpose of determining the influence of various nutritional relations, of hydrogen-ion concentration, of temperature, and of other factors upon the germination of the spores of certain species of three families of the Basidiomycetae, the Agaricaceae, Nidulariaceae, and Lycoperdaceae.

The earliest attempts at germinating the spores of fungi are generally credited to PREVOST in 1807, EHRENBURG in 1821, CORDA in 1842, and to others. HOFFMANN's work (14), however, published in 1860, was the earliest extensive investigation of this character and seems to mark the initiation of the use of the hanging drop technique, which, improved upon by others, has gradually developed into a standardized procedure centering around the Van Tieghem cell. HOFFMANN's results were so definite that he was able to demonstrate the effect of environmental factors and of internal factors, such as age, upon the germination of the spores; but he was unable to induce the germination of the spores of the Lycoperdaceae. BREFELD (3) and DE BARY (8) found the same negative response a few years later.

Decoctions of manure, fruits, and other materials were the favored media of those studying the germination of spores during this early period, the use of media of known composition coming later. DUGGAR (9) used a variety of media, and observed that glycerin as a source of carbon yields higher germination percentages of the spores of certain saprophytic fungi than does sugar, and that neutral salts give greater stimulation than do acid ones.

FERGUSON (11) studied the effect of types of nutrition, reaction of medium, temperature, light, and age upon the germination of the spores of *Agaricus campestris* and many other species of saprophytic Basidiomycetes. She succeeded in securing germination percentages

as high as 85 per cent, and demonstrated that temperature extremes constitute the only special stimulus required to induce germination in some instances. FERGUSON also attempted to induce germination of puffball spores, but without success. SWARTZ (18) reports that COOL also failed to germinate the spores of several species of puffballs. SWARTZ himself claims to have germinated the spores of *Calvatia saccata* and of *Lycoperdon pyriforme* after exposing them to alternate wetting and drying.

From about 1904, the majority of workers have attacked the problem of germination of spores of certain refractory saprophytic Basidiomycetae by determining the influence of the digestive system of animals upon their capacity for germination (13, 1, 4, 5). STYER (17) and BECHMANN (2) confined their investigations chiefly to studying the influence of nutritional and other physiological factors upon the germination of the spores of the commercially important mushroom, *Agaricus campestris*.

Of special interest in this connection is the early recognition of the importance of the reaction of the medium to the germination of fungous spores, culminating in the quantitative studies of WEBB (19) and others. FINDLAY (12), following up the earlier work of FALCK, secured positive results with *Merulius lachrymans* only in acid media, while EVANS and HARRAR (10) showed the influence of pH in the germination of the oospores of *Sclerospora graminicola*.

### Technique

SPORES.—The spores used in this work were aseptically removed from the fruiting bodies of the following species of Basidiomycetae collected in and about the city of Madison, Wisconsin: *Coprinus micaceus* (Bull.) Fr.Cke., *C. comatus* (Fl. Dan.) Fr.Cke., *Lepiota cepaestipes* (Sow.) Fr., and *Armillaria mellea* (Vahl) of the Agaricaceae; *Cyathus striatus* (Huds.) Pers. and *Cyathus olla* (Batsch) Pers. of the Nidulariaceae; and *Bovistella radicata* (Mont.) Pat. and *Calvatia cyathiformis* (Bosc) Morg. of the Lycoperdaceae.

CULTURES.—Two types of cultures were used, namely, the Van Tieghem hanging drop culture and a petri dish type of culture. A detailed description of the technique employed in mounting and using the Van Tieghem cell is given by CLARK (6).

The petri dish technique was developed to facilitate the use of large numbers of cultures. Into each 100 mm. petri dish there was placed a piece of filter paper 9 cm. in diameter, moistened with the particular culture solution to be employed. The culture drops were disposed each on a cover slip, the spores were sown, and the cover slips arranged on the moist filter paper, covered, and incubated. For microscopic examination of the spores in the drop the cover slip was aseptically removed from the petri dish and inverted over a mounted glass ring. When in use, the petri dishes were kept in metal cans each provided with a convenient basket for removal of the dishes and moist paper towelling for maintaining humidity.

**SOWING OF SPORES.**—To minimize clumping tendencies, all spore suspensions were made in a physiological salt solution consisting of: 3.0 gm. sodium chloride, 0.2 gm. anhydrous calcium chloride, and 0.2 gm. potassium chloride to a liter of distilled water. Spores were transferred to this from the spore print with a platinum loop in the proportion of one loopful of spores to every cubic centimeter of the salt solution.

In suspending the spores of the Nidulariaceae, the peridioles were sterilized for 10 minutes in 20 per cent calcium hypochlorite, washed off with sterile distilled water, transferred to the sterile salt solution, and there crushed against the inner walls of the test-tubes with a sterile glass rod, thus liberating the spores.

Transfer of the suspension to culture drops was made with capillary pipettes, each flanged at one end to fit snugly into a rubber nipple and drawn out at the other end to a diameter of  $250\ \mu$  and a length of 8 cm. This size delivers spores in such quantities as to provide an average of about 60 spores to the microscopic field of the high-power objective. Transfer of culture solution to cover slips was made with pipettes 15 cm. long and fire polished at one end to deliver small drops of uniform size and shape.

**GENERAL TECHNIQUE.**—All of the preceding operations were conducted inside a large transfer chamber, which was thoroughly sponged on the inner sides with a strong mercuric bichloride solution before being used. As a precaution against dust, a constant supply of steam was provided by boiling water inside the chamber. In addi-

tion, the usual bacteriological precautions were rigorously observed and no difficulty with contaminations was experienced.

Unless otherwise stated, incubation was in the dark and at a constant temperature of 28° C.

All glassware was cleansed with special care, first being boiled in a weak ammoniacal solution, then in a sulphuric acid-chromate solution, and finally in distilled water. Sterilization of dry glassware was accomplished by a two-hour exposure in a hot air oven at 170° C.

TABLE I

SPORE GERMINATION IN A STANDARD NUTRIENT SALT SOLUTION OF PH 4.6

INCUBATION IN HOURS	TOTAL NO. OF SPORES	GERMINATION			LENGTH OF GERM TUBE	
		No.	PERCENTAGE	AVERAGE (PER CENT)	MICRONS	AVERAGE IN MICRONS
288	29	2	9.09	8.16	19.47	14.75
	17	1	5.88		17.70	
	42	4	9.52		7.08	
384	40	2	5.00	7.95	42.48	51.92
	19	2	10.52		10.62	
	48	4	8.33		102.66	
508	55	5	9.09	11.75	10.62	12.98
	52	5	3.84		14.16	
	72	16	22.22		14.16	
Average.....	.....	.....	.....	9.28	.....	26.55

In making observations of the germination in drop cultures, three readings were taken at each inspection, and measurements of the lengths of germ tubes were made with a high-power objective and an ocular micrometer. Each reading was taken from a definitely different field of the drop and consisted of observing the total number of spores, the number of germinated spores, and the length of a germ tube taken at random. In general, the whole procedure is more readily exhibited by a sample protocol (table I), giving the germination of the spores of *Coprinus micaceus* in one of the 19 general culture solutions.

A spore was considered germinated when a germ tube of at least

1.77  $\mu$  had formed, this length corresponding to one-half unit of the scale on the ocular micrometer used. All germination percentages given in the results are actual. The practice of expressing the highest percentages as 100 per cent and adjusting other results accordingly was avoided in this investigation.

### Investigation

#### I. GERMINATION OF SPORES OF CERTAIN AGARICACEAE AND NIDULARIACEAE

Nineteen culture solutions were first tested for their value in stimulating germination of the spores of *Coprinus micaceus* and *C. comatus*. Of these media, three were selected as best suited for investigating the effect of hydrogen-ion concentration upon spore germination. Then the influence of temperature was determined, using the most effective medium at optimum hydrogen-ion concentration for the germination of the spores of each of the six organisms used.

A. SPORES OF *COPRINUS MICACEUS* AND *C. COMATUS* IN THE NINETEEN MEDIA.—The three culture solutions selected for further germination studies are the 0.5 per cent glycerin solution, the 4 per cent sucrose solution, and the beef broth. The reasons for this selection are apparent from a study of the results secured during an incubation period of 672 hours and are recorded in table II. The beef broth solution and the 4 per cent sucrose solution yielded the highest germination percentages for the spores of *Coprinus micaceus*; and the 0.5 per cent glycerin solution and 4 per cent sucrose solution, the highest for the spores of *C. comatus*.

Drawing conclusions from results secured with 19 solutions of varying strengths and of varying hydrogen-ion concentrations is unwise, yet one tendency which seems to be indicated in this preliminary test must be mentioned, that hydrogen-ion concentrations between pH 5.9 and 7.7 appear to favor germination of the spores of *Coprinus micaceus*; and concentrations between pH 5.9 and 6.7, germination of the spores of *C. comatus*.

B. EFFECTS OF HYDROGEN-ION CONCENTRATION ON SIX SPECIES OF SPORES.—A series of 15 solutions ranging in hydrogen-ion concentration from pH 3.0 to 10.0 was prepared from each of the three culture

solutions selected: the beef broth solution, the 4 per cent sucrose solution, and the 0.5 per cent glycerin solution. The series was prepared by the use of determined quantities of  $N/5$   $H_3PO_4$  and  $N/10$   $NaOH$ . The petri dish method was employed in order to accommodate this large number of tests.

TABLE II

SUMMARY OF GERMINATION PERCENTAGES AND GERM TUBE GROWTH OF SPORES OF *COPRINUS MICACEUS* AND OF *C. COMATUS* CULTURED IN GENERAL CULTURE SOLUTIONS\*

CULTURE SOLUTIONS	pH	C. MICACEUS		C. COMATUS	
		PER-CENTAGE	TUBE GROWTH IN MICRONS	PER-CENTAGE	TUBE GROWTH IN MICRONS
Nutrient solution	4.6	9.28	26.55	0.00	0.00
Beef broth	6.9	26.60	44.65	2.40	19.47
2% peptone	7.7	22.82	51.40	0.00	0.00
0.5% egg albumin	6.5	22.08	35.84	0.00	0.00
2% glucose	5.9	17.24	37.02	7.64	97.99
4% sucrose	5.9	27.23	43.41	10.11	30.85
0.5% glycerin	6.7	22.17	41.44	14.62	25.87
1% potato starch	6.4	16.36	34.93	0.00	0.00
Acetic acid	3.7	12.13	96.72	0.00	0.00
Oxalic acid	3.3	8.71	48.38	0.00	0.00
Citric acid	3.5	3.47	20.94	2.30	46.12
Hydrochloric acid	2.9	2.47	37.85	0.00	0.00
0.5% ammonium tartrate	6.3	15.81	32.92	2.62	12.53
0.5% calcium phosphate	6.5	17.62	36.87	6.73	43.66
0.5% sodium carbonate	10.0	8.59	21.41	6.11	101.63

\* No germination whatever occurred in tartaric acid, pH 3.4; glutamic acid, pH 3.1; sulphanilic acid, pH 2.3; and sulphuric acid, pH 2.9.

The results secured, summarized briefly in table III and graphically by figure 1, indicate at least four types of spore germination responses to variations in the hydrogen-ion concentration of the culture solution.

First, with the exception of the spores of the two species of *Coprinus*, the hydrogen-ion range in which germination of the spores of the Agaricaceae and Nidulariaceae will occur, under the conditions of these experiments, is between pH 5.0 and 8.5. In the case of *Cyathus olla* the range is even narrower, germination having occurred only between pH 6.0 and 8.5. As noted, the spores of *Coprinus co-*



TABLE III

SUMMARY OF GERMINATION PERCENTAGES AND GERM TUBE GROWTH OF SPORES  
OF SIX SPECIES IN THREE SERIES OF CULTURE SOLUTIONS RANGING IN  
HYDROGEN-ION CONCENTRATION FROM pH 3.0 TO 10.0

pH	BEEF BROTH		0.4% SUCROSE		0.5% GLYCERIN		BEEF BROTH		0.4% SUCROSE		0.5% GLYCERIN	
	PER-CENT-AGE	TUBE GROWTH (μ)	PER-CENT-AGE	TUBE GROWTH (μ)	PER-CENT-AGE	TUBE GROWTH (μ)	PER-CENT-AGE	TUBE GROWTH (μ)	PER-CENT-AGE	TUBE GROWTH (μ)	PER-CENT-AGE	TUBE GROWTH (μ)
<i>Coprinus micaceus</i>						<i>Coprinus comatus</i>						
3.0	0.00	.....	0.00	.....	0.00	37.37	0.00	.....	0.00	.....	4.13	27.73
3.5	0.00	.....	0.00	.....	5.11	44.05	0.00	.....	0.00	.....	4.72	30.09
4.0	0.00	.....	0.00	.....	5.26	44.45	0.00	.....	0.00	.....	6.08	31.86
4.5	11.87	20.53	0.00	.....	5.73	58.29	0.00	.....	0.00	.....	6.85	30.97
5.0	12.18	42.88	0.00	.....	7.88	167.86	0.00	.....	0.00	.....	7.77	41.30
5.5	8.89	149.11	0.00	.....	13.48	186.05	0.00	.....	0.00	.....	12.26	44.30
6.0	17.25	178.88	0.00	.....	17.58	212.40	5.59	15.34	3.54	14.45	18.17	56.98
6.5	18.81	71.51	8.14	50.74	10.03	212.40	7.50	20.55	12.22	24.48	22.15	74.04
7.0	27.66	210.67	17.10	67.85	28.93	194.70	11.97	34.21	4.69	18.58	23.03	207.38
7.5	31.89	212.40	6.84	30.97	17.29	167.56	6.50	29.20	4.32	15.04	35.40	212.40
8.0	29.12	212.40	6.89	27.14	15.60	167.56	1.87	9.44	0.00	.....	31.45	155.40
8.5	13.71	176.25	4.35	25.57	14.66	97.15	0.00	.....	0.00	.....	26.65	122.13
9.0	10.77	106.64	2.26	21.83	15.44	77.49	0.00	.....	0.00	.....	23.62	114.16
9.5	15.39	75.05	2.38	11.21	13.79	43.27	0.00	.....	0.00	.....	13.27	53.39
10.0	10.47	25.07	1.93	9.44	5.63	28.32	0.00	.....	0.00	.....	6.39	25.66
<i>Lepiota cepaestipes</i>						<i>Armillaria mellea</i>						
5.0	4.75	13.27	0.00	.....	0.00	.....	7.61	14.76	5.59	11.01	2.32	7.38
5.5	5.97	24.19	3.75	12.98	0.00	.....	9.80	20.65	6.68	10.62	2.01	6.29
6.0	9.09	24.39	4.73	16.91	2.96	8.86	6.44	57.82	8.56	23.45	4.02	9.44
6.5	11.60	53.10	6.00	22.71	4.49	12.98	6.43	28.81	10.99	15.04	4.57	10.03
7.0	11.71	33.51	4.66	23.89	4.86	20.35	8.57	20.26	4.57	15.04	6.53	13.57
7.5	0.00	.....	4.30	14.16	6.68	27.14	0.00	.....	5.45	12.39	5.43	12.98
8.0	0.00	.....	3.44	12.29	5.27	22.03	0.00	.....	4.36	11.06	3.16	10.89
8.5	0.00	.....	0.00	.....	5.45	21.83	0.00	.....	0.00	.....	1.90	10.62
<i>Cyathus olla</i>						<i>Cyathus striatus</i>						
5.0	0.00	.....	0.00	.....	0.00	.....	0.00	.....	0.00	.....	2.66	13.27
5.5	0.00	.....	0.00	.....	0.00	.....	0.00	.....	4.48	16.52	4.21	21.83
6.0	0.00	.....	3.28	6.09	4.84	18.29	0.00	.....	5.98	24.32	5.15	31.47
6.5	5.97	43.07	4.41	9.05	5.34	31.27	7.24	48.38	8.09	25.37	6.05	36.87
7.0	9.23	77.88	4.34	29.20	4.84	21.83	6.94	33.82	8.42	34.81	8.62	64.90
7.5	7.92	62.77	5.28	43.61	4.04	15.14	5.40	55.46	7.58	22.42	8.69	46.61
8.0	16.24	56.64	3.57	16.52	3.56	10.62	0.00	.....	6.04	18.88	7.35	37.37
8.5	0.00	.....	2.78	14.16	0.00	.....	0.00	.....	0.00	.....	5.32	27.14

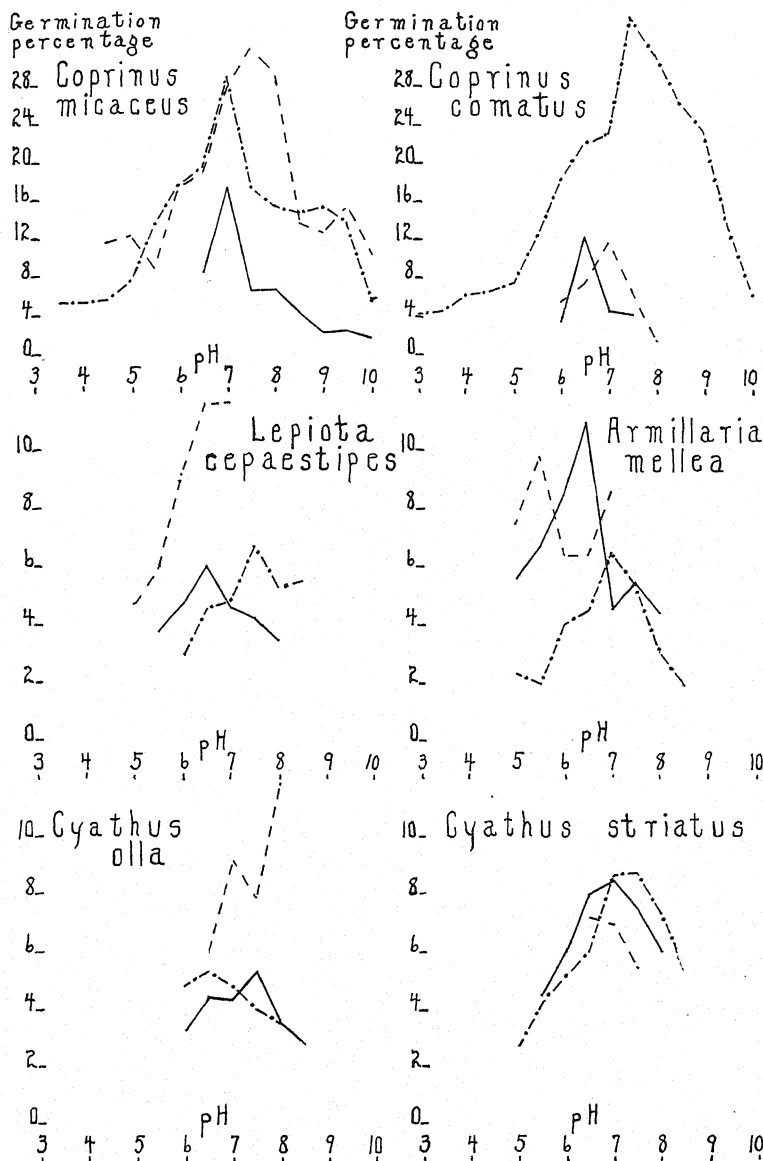


FIG. 1.—Effect of hydrogen-ion concentration upon germination percentage of spores of the six species cultured in beef broth, 4 per cent sucrose, and 0.5 per cent glycerin solutions: beef broth — — — —; 4 per cent sucrose ———; 0.5 per cent glycerin — · — · — ·.

*matius* germinated at all pH values in glycerin, and the spores of *C. micaceus* in all except pH 3.0.

Second, the general optimum pH for germination of the spores of the Agaricaceae and of the Nidulariaceae is indicated in these studies as 7.5. It is a little lower for two forms, namely, pH 7.0 for the spores of *Lepiota cepaestipes* and pH 6.5 for the spores of *Armillaria mellea*. Offsetting these, however, is the slightly higher pH value at which the spores of *Cyathus olla* germinate best, pH 8.0.

Third, the effect of the hydrogen ions and the hydroxyl ions upon spore germination varies. Germination of the spores of the two species of *Coprinus* and of the spores of *Cyathus olla* was more extensive on the alkaline side, and therefore can be said to have been favored by an excess of hydroxyl ions over hydrogen ions and somewhat inhibited by the opposite. Just the reverse is true of the germination of the spores of the other species; hence it appears that the effect of hydrogen ions or of hydroxyl ions upon germination of mushroom spores varies with the species.

Fourth, with the exception of *Coprinus*, the optimum hydrogen-ion concentration for germ tube development is in general slightly below the hydrogen-ion concentration optimum for germination.

Of the media used in this study the following were selected as being most favorable for spore germination: pH 7.5 beef broth for the spores of *C. micaceus*; pH 7.5 0.5 per cent glycerin for the spores of *C. comatus*; pH 7.0 beef broth for the spores of *L. cepaestipes*; pH 6.5 sucrose for the spores of *A. mellea*; pH 8.0 beef broth for the spores of *C. olla*; pH 7.5 0.5 per cent glycerin for the spores of *C. striatus*.

C. EFFECTS OF TEMPERATURE ON SIX SPECIES OF SPORES.—Spores of the same six species of the Basidiomycetae used in the hydrogen-ion tests were also used to determine the effect of temperature upon spore germination. The spores were cultured in the solution found best suited as a result of the preceding hydrogen-ion tests. Incubation was at ten temperatures ranging from 0° to 45° C. and arranged for by the use of a series of electrically controlled refrigerators and incubators. Lack of time made it impossible to examine each culture more than three times. Hence the results secured are lower than those in the hydrogen-ion test, but just as reliable, since

results of the hydrogen-ion test developed within 196 hours, the incubation period allowed for the temperature tests.

The results secured are summarized briefly in table IV, graphically by figure 2, and indicate the following tendencies.

The general optimum temperature for the germination of the spores under the conditions of this investigation can safely be stated as 30° C., the one exception being that of *C. micaceus*, with a maximum in these tests at 35° C. It is evident from this that higher germination percentages would have been secured by others investigat-

TABLE IV

SUMMARY OF GERMINATION PERCENTAGES AND GERM TUBE GROWTH OF SPORES OF THE SIX SPECIES CULTURED AT SEVEN DIFFERENT TEMPERATURES IN THE SOLUTION FOUND BEST FOR EACH SPECIES

TEMPERATURE (°C.)	COPRINUS MICACEUS		COPRINUS COMATUS		LEPIOTA CEPAESTIPES		ARMILLARIA MELLEA		CYATHUS OLLA		CYATHUS STRIATUS	
	PER- CENT- AGE	TUBE GROWTH (μ)	PER- CENT- AGE	TUBE GROWTH (μ)	PER- CENT- AGE	TUBE GROWTH (μ)	PER- CENT- AGE	TUBE GROWTH (μ)	PER- CENT- AGE	TUBE GROWTH (μ)	PER- CENT- AGE	TUBE GROWTH (μ)
15	1.72	212.40	3.37	33.63	0.00	.....	0.00	.....	0.00	.....	0.00	.....
20	3.30	23.99	6.72	66.08	0.00	.....	0.00	.....	2.59	8.45	1.99	10.82
25	15.09	69.33	12.69	87.02	3.23	10.42	3.39	11.41	4.26	10.12	3.93	18.08
30	15.05	113.18	17.18	171.98	8.10	34.22	8.97	30.68	10.52	31.47	7.65	44.05
35	77.73	212.40	16.29	189.09	5.12	27.73	2.71	7.28	4.53	16.32	3.19	18.09
40	39.54	212.40	14.38	97.05	2.54	8.26	0.00	.....	0.00	.....	0.00	.....
45	27.48	156.94	7.58	44.84	0.00	.....	0.00	.....	0.00	.....	0.00	.....

ing spore germination had incubation temperatures been raised above the more popular 28° or 30° C., at least for species of *Coprinus*.

As to the temperature range, the widest adaptability was that manifested by the spores of *Coprinus*, which germinated at from 15° to 45° C. The narrowest range was that of the spores of *Armillaria mellea*, which did not germinate at a temperature below 25° C. nor at one above 35° C.

The temperature best for highest germination percentage is generally also optimum for longest germ tube development. The exception to this appears with the spores of the species of *Coprinus*, which developed longest germ tubes at a temperature 5° higher than the optimum for greatest germination percentage.

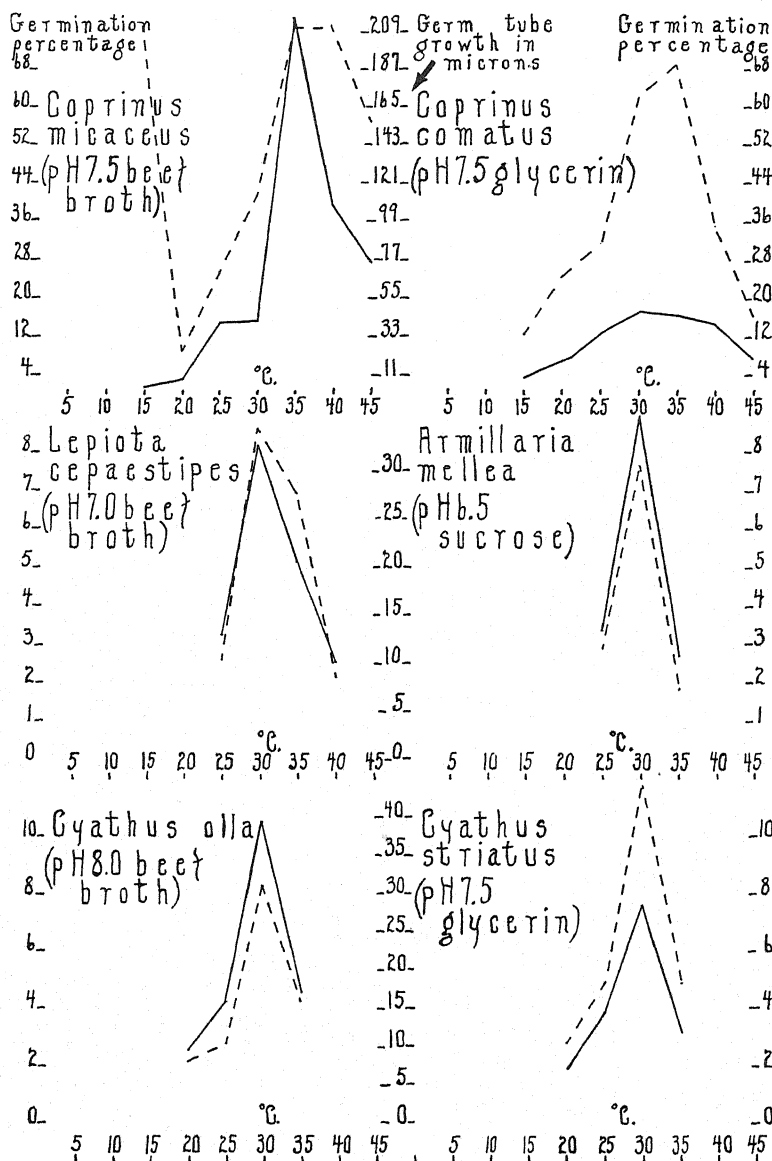


FIG. 2.—Effect of temperature upon germination percentage and upon germ tube growth of spores of the six species in the solution indicated for each: germination percentage —————; germ tube growth — — — — —.

## II. GERMINATION EXPERIMENTS WITH SPORES OF CERTAIN LYCOPERDACEAE

A. SPORES OF *BOVISTELLA RADICATA* AND *CALVATIA CYATHIFORMIS*.—Of the 25 culture solutions used, 19 were the same as those used in the preliminary test of the first part of this paper. The other six solutions were weak infusions of well rotted pig dung, horse dung, and sheep dung, and weak infusions of sludge, silt, and Vigoro. Vigoro is a commercial preparation of 4.0 per cent nitrogen, 12.0 per cent available phosphoric acid, and 4.0 per cent water-soluble potash.

Despite an incubation period of 1200 hours for all solutions, no germination whatever was observed. Hence it was thought advisable to restrict further investigation to just one species of the Lycoperdaceae. The spores of *Bovistella radicata* were selected because a greater supply of them had been collected than of other forms.

B. EXPOSURE OF SPORES OF *BOVISTELLA RADICATA* TO "NATURAL" CONDITIONS.—Of the conditions probably prevailing about these spores as they germinate in nature, those tested were alternate wetting and drying as suggested by SWARTZ (18), alternate freezing and thawing, and exposure to extreme humidity.

Alternate wetting and drying of spores was accomplished with three petri dish cultures having ten cover slips each and a drop of distilled water, inoculated with the spores, on each cover slip. One of these cultures was incubated at 25°, a second at 30°, and a third at 34° C. The drops evaporated very rapidly and were replaced with sterile distilled water daily. This was continued for 17 days. Beginning with the eighth day, however, a cover slip with its inoculated drop was removed from each petri dish, transferred to a Van Tieghem cell, and incubated at 28° C. This was repeated daily until all 30 cover slips had been transferred to Van Tieghem cells. No germination occurred during the time allowed, 792 hours.

Spores were given freezing and thawing treatments by daily exposures of 4 hours to low temperatures ranging from -18° C. at the beginning of the period to 0° C. at the conclusion. At other times these spores were kept at 27° C. This procedure was followed on each of 8 days, when the spores were cultured in distilled water, nutrient salt solution, 7 per cent sucrose pig dung infusion, and physiological

salt solution. Germination did not occur even after 672 hours of incubation.

Spores were subjected to conditions of extreme humidity by seeding them on cover slips placed in the lower shells of petri dishes that were supported over water in a humidity chamber. But after 826 hours of this type of exposure no traces of germination had appeared.

C. EXPOSURE OF SPORES OF *BOVISTELLA RADICATA* TO AGENTS INTENDED TO INCREASE SPORE MEMBRANE PERMEABILITY. —This third phase was based on the possibility that delayed germination of these spores may primarily be due to impermeability of the spore membranes to stimulative and nutritive substances. Efforts to overcome this impermeability consisted of exposing the spores to change of osmotic pressure, low temperature, high temperature, enzymes, anaesthetization, and various combinations of these.

Exposing the spores to greater osmotic pressure was accomplished by culturing them in drops of the six infusions to each of which 7 per cent sucrose had been added. Germination in these solutions did not occur even after an incubation period of 936 hours.

Very low temperature was made possible through the use of liquid air secured in a thermos flask into which hard glass test-tubes containing spore masses were lowered successively for the varying periods of 2, 5, 10, 30, and 60 minutes. Following these exposures the spores were cultured in distilled water, physiological salt solution, nutrient salt solution, 7 per cent sucrose pig dung infusion, 7 per cent sucrose horse dung infusion, Troemmer's malt extract solution containing enzymes from a 7 months' old culture of *Aspergillus niger*, and nutrient salt solution containing enzymes from a 7 days' old culture of *A. niger*. An incubation period of 816 hours was allowed but germination did not occur.

Two methods were undertaken to test the effect of high temperature upon the germination of the spores. One was to incubate the spores in culture media at temperatures of 30°, 34°, 38°, and 42° C. The media used were the 2 per cent glucose solution and the oxalic acid solution. Spore germination did not occur during an incubation period of 1320 hours.

The other method was to expose spores to 100° C. for periods of 5 and 10 minutes. Moreover, the 5 minutes' exposure to 100° C. was

given the spores which had previously received the alternate thawing and freezing treatment, the spores which had been exposed to the temperature of liquid air for 10 minutes, and those which had been exposed to the temperature of liquid air for 30 minutes. Further, these spores were then cultured in distilled water, in 7 per cent sucrose horse dung infusion, and in young enzyme solution. This combination of treatments yielded no spore germination during an incubation period of 768 hours.

Ethylene chlorohydrin was selected for attempting to increase spore membrane permeability by anaesthetization. The spores were treated in test-tubes. Exposures were of two periods, 15 minutes and 30 minutes, daubers holding cotton saturated with ethylene chlorohydrin being withdrawn at the end of these periods and replaced with cotton plugs. This treatment was also given spores which had previously been subjected to alternate thawing and freezing, spores which had been exposed to the temperature of liquid air for 10 and 30 minutes respectively, and spores which had been subjected both to the temperature of liquid air for 10 minutes and that of 100° C. for 5 minutes. These spores were then cultured in distilled water, in 7 per cent sucrose horse dung infusion, and in young enzyme solution. Some of the spores therefore received the combined effect of low temperature, high temperature, anaesthetization, and maceration by enzymes. However, germination did not occur during an incubation of 768 hours.

### Summary

As a result of these researches, and granted the conditions of this investigation, the following conclusions seem justified.

1. Three culture solutions in which a high percentage of the spores of *Coprinus micaceus* and of *C. comatus* will germinate are beef broth, 4 per cent sucrose, and 0.5 per cent glycerin.

2. With the exception of the spores of the species of *Coprinus*, the hydrogen-ion range in which the germination of the spores of the Agaricaceae and Nidulariaceae will occur is between pH 5.0 and 8.5. The spores of *C. comatus* germinate over a range of from pH 3.0 to 10.0, and those of *C. micaceus* over a range of from pH 3.5 to 10.0.



3. The general optimum pH for germination of the spores of the Agaricaceae and of the Nidulariaceae is pH 7.5.

4. The effect of the hydrogen ions and of the hydroxyl ions upon the germination of the spores of the Agaricaceae and the Nidulariaceae varies with the species.

5. The optimum hydrogen-ion concentration for germ tube development is slightly below the hydrogen-ion concentration optimum for germination.

6. The general optimum temperature for the germination of the spores of the Agaricaceae and of the Nidulariaceae is 30° C.

7. The widest temperature range under which germination will occur is from 15° to 45° C. for the spores of *Coprinus micaceus* and of *C. comatus*. The general range is from 20° to 40° C. for the spores of the other species of Agaricaceae and Nidulariaceae.

8. Up to the time of this writing, efforts at germinating the spores of *Bovistella radicata* and of *Calvatia cyathiformis* in artificial media have been unsuccessful.

9. In this investigation, the germination percentages of the spores of *Coprinus comatus* are higher than all other results thus far reported for this species.

10. This is the first definite investigation reported of the germination of the spores of four of the species used, namely, *Lepiota cepaestipes*, *Armillaria mellea*, *Cyathus olla*, and *Cyathus striatus*.

The writer is indebted to DR. B. M. DUGGAR for his suggestion of this problem and for his kind and valuable advice during the progress of the investigation and preparation of this report.

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# A STUDY OF THE VEGETATIVE PHASES OF EPHEDRA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 457

PAUL D. VOTH

(WITH TWENTY-FIVE FIGURES)

## Introduction

According to STAPF (15), species of *Ephedra* are found in the drier, warmer regions of every continent except Australia. The genus has received, together with *Gnetum* and *Welwitschia*, much attention in phylogenetic studies. The value of certain Asiatic species of *Ephedra* as a source of ephedrine, and of other species growing in the arid southwestern part of the United States as browse for cattle, sheep, and goats (6), has increased the economic interest in the genus.

The germination of *Ephedra* has been described in some detail by STRASBURGER (17) and by BOWER (4). Both stress the fact that *Ephedra* differs from *Gnetum* and *Welwitschia* in that no lateral projection of the hypocotyl (foot) is present in the former. BOWER (4) postulates that the suspensor and the living remains of the nucellus may act as conductors of nutrient material from the megagametophytic tissue to the seedling. SCHACHT (14) as well as HILL and DE FRAINE (10) show *Ephedra* seedlings with a structure that appears to be a foot. MARKGRAF (12), although he illustrates some of the stages of germination after HILL and DE FRAINE, correctly pictures the seedling without a feeding organ.

STRASBURGER (17) was the first to describe the root-stem transition in the hypocotyl of *Ephedra*. He pointed out that the vascular bundles in the hypocotyl follow a course similar to that followed by the bundles in the hypocotyl of *Araucaria*. The only other description of the vascular system in the hypocotyl of *Ephedra* is given by HILL and DE FRAINE (10). In their illustration a semicircular girdle of transfusion tissue connects the two hypocotyledonary bundles at a distance considerably below the cotyledons. No such condition was found in the species under investigation.

Apparently no detailed studies have been made to determine the vascular situation in the epicotyl of an *Ephedra* seedling. The course of the vascular bundles in the stem tip and the larger branches of plants past the seedling stage has been the subject of investigation since the time of NÄGELI (13). His longitudinal diagram of the stem of *E. vulgaris* roughly agrees with the situation in the stem of *E. pedunculata*, the species upon which the greater part of the present paper is based. GEYLER (9) extended the observations of NÄGELI to *E. equisetiformis* Webb and Berth., to *E. helvetica* C. A. Meyer (*E. distachya* Gand.), and to *E. campylopoda* C. A. Meyer (*E. distachya* Durv.), all of which were in accord with the species described by NÄGELI. STRASBURGER (17, pp. 77-78) reported that *E. altissima* does not have the customary eight bundles in the stem but only six, owing to the non-divergence of a pair of bundles in the second internode below the node at which the bundles diverge into the leaves. The vascular bundles in the stem continue for two internodes before they diverge into the opposite leaves. DE BARY (1) reviewed the vascular condition in the different species of *Ephedra* and referred to the work of STRASBURGER (17) on *E. campylopoda*, who showed that a "complementary" bundle is differentiated parallel to and between the two foliar bundles in the internode just below the node at which the foliar bundles diverge into the leaves. The "complementary" bundle evidently is lost in the girdle of xylem which is found at every node. This explains the occurrence of ten bundles in the stem of this species.

The number of bundles in the stem of *Ephedra* has been made one of the diagnostic characteristics of a new Asiatic species described by STAPF (16). LIU (11) has since shown that the number (eight) mentioned by STAPF is not constant, and that as many as ten bundles are found in the older parts of the plant.

STRASBURGER (17) and BERTRAND (2) have compared the anatomical features of *Ephedra* and the other Gnetales with those of the Coniferales. EVANS (7) described the anatomical features of several North American species of *Ephedra*, emphasizing the xeromorphic structures of stem and leaf. More recently GEORGE (8) has concluded that anatomically *Ephedra* is gymnospermous in its affinities. COULTER and CHAMBERLAIN (5) have discussed some of the anatom-

ical features of the Gnetales in relation to phylogeny. BOODLE and WORSDELL (3) compared the anatomy of *Ephedra* with that of *Casuarina*.

### Materials and methods

Seeds and vegetative portions of *Ephedra pedunculata* Englm. were collected about 8 miles southeast of Lubbock, Texas, in May and June, 1931 and 1932. The vegetative parts were preserved in a formalin-alcohol solution containing 6 per cent commercial formalin and 60 per cent of 95 per cent alcohol. The seeds with their surrounding red, fleshy involucre bracts were air dried and later germinated in the University of Chicago greenhouses.

Most phylogenetic studies on *Ephedra* have been made on plants old enough to have lost many of their primary features. This paper records some observations on the primary stelar condition of the seedling.

Stem tips of *E. viridis* Coville, also a North American species inhabiting the arid southwest, and of *E. altissima* Desf. (?), a North African species, were used for comparison.

Seedlings used for the study of the root-stem transition were 33 days old; those of the epicotyl, 51 days. Formalin-alcohol (6-60 per cent), Flemming's medium, or Sax's modification of Nawaschin's fluid were used as fixing agents. Serial sections were cut 12  $\mu$  thick.

### Investigation

#### SEEDLING

Under favorable conditions the seeds germinate in about ten days. The hypocotyl elongates rapidly and in two to five days the primary root, devoid of laterals, has grown two or three inches. Concurrently the cotyledons elongate, especially in their basal portions. The seed coat either remains in the substratum and the two cotyledons (bent as a knee in their basal portion) emerge completely, or the seed coat is brought above ground by the cotyledons, their tips still in position to absorb food from the remaining tissue of the megagametophyte. After a short period of growth the upper part of the hypocotyl straightens out. In the greenhouse, in direct light, the cotyledons average 5 cm. in length and 1 mm. in diameter. They are linear in

outline and in cross-section are roughly semicircular, their flattened sides facing each other and the stem. The cotyledons function for a time as photosynthetic organs.

At about 15 mm. below the divergence of the cotyledons the axis is definitely recognizable as primary root. This portion of the axis is smaller in diameter than the hypocotyl. The cortex, which is soon crushed, becomes darkened.

The diameter of the axis is much greater at the level of the divergence of the cotyledons, the bases of which form a cotyledonary sheath about 1.5 mm. in length.

The leaves are opposite, in decussate arrangement. Near the growing point the internodes are very short, but when mature they average 3 cm. in length. During the early life of the seedling, when the primary root continues to grow and the lateral roots are formed, growth of the epicotyl is very slow; later the rate of elongation is largely dependent upon water supply.

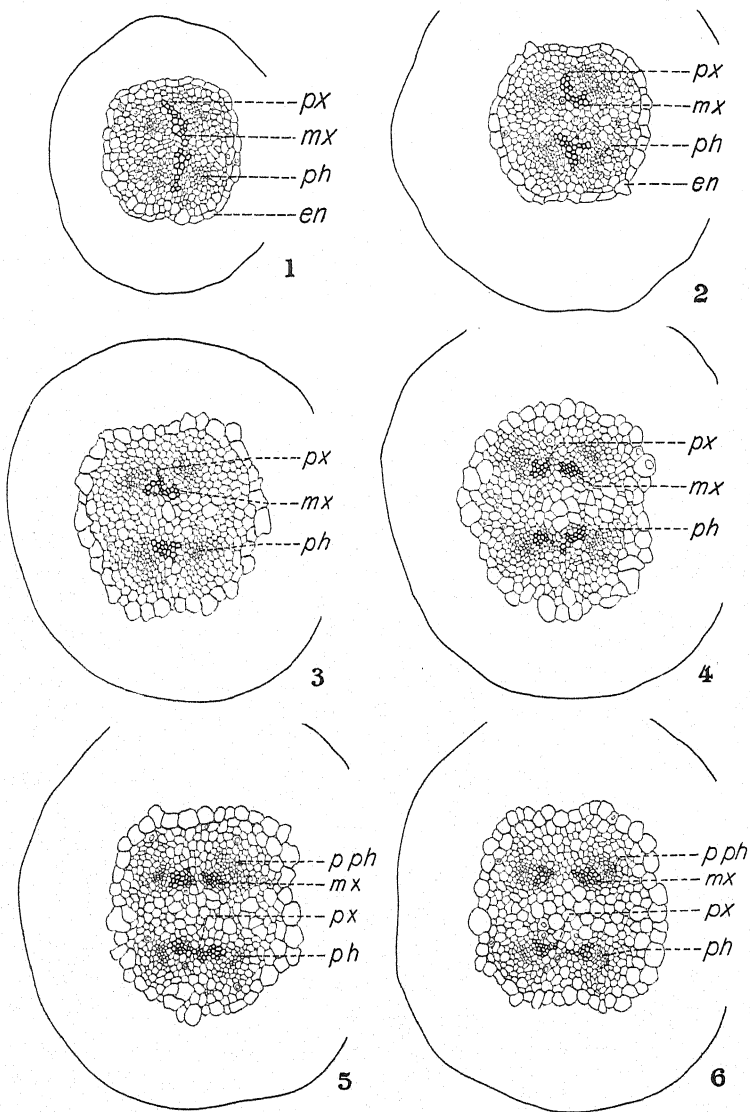
In the greenhouse the leaves of *Ephedra* remain functional for more than a year, but in the arid native habitat they become dry and scalelike within a few weeks. The stem functions as a photosynthetic organ until secondary growth and weathering disrupts the palisade layers in the cortex.

As will be seen from figures 21 and 22, buds can form in the axils of the cotyledons. Such buds, as well as those in the axils of the leaves, become active when the stem tip above them is clipped or grazed off. More than one bud may form in an axil, which produces the whorled appearance of branches of the older native plants.

#### PRIMARY ROOT AND ROOT-STEM TRANSITION

The primary root is diarch, exarch. In some roots (fig. 1) a few cells in the center of the axis remain parenchymatous, in others the protostelic condition is attained only in the more distal portions.

In seedlings a month old the protoxylem elements are spiral or modifications of this general type. At places bordered pits occur on these elements. These pits are more numerous on walls where two tracheids are adjoined, but they also occur on walls which border on parenchyma cells. The walls of the metaxylem tracheids which are differentiated first (abaxial) are reticulate, while those differentiated



FIGS. 1-6.—Transverse sections of primary root and hypocotyl of a seedling 33 days old (only stele and endodermis shown in detail): Fig. 1, cross-section through primary root just below hypocotyl region, showing four phloem groups (*px*, protoxylem; *mx*, metaxylem; *ph*, phloem; *en*, endodermis). Figs. 2, 3, cross-sections through lower part of hypocotyl. Metaxylem is being differentiated abaxially. Figs. 4-6, cross-sections of hypocotyl showing tangential divergence of each primary xylem group (*p ph*, proto-phloem).  $\times 47$ .

later are more heavily thickened and possess bordered pits with slit openings and tertiary thickenings. The innermost metaxylem tracheids do not mature until secondary growth has begun. At no level are more than four protoxylem elements visible at a protoxylem point.

The phloem of the primary root is difficult to distinguish. Several millimeters below the hypocotyl two phloem groups alternating with the two xylem groups are visible in cross-section. Near the hypocotyl four groups of elongated cells of small diameter, each with an elongated nucleus, are discernible lateral to the primary xylem groups (fig. 1); hence each xylem strand is flanked on either side by a phloem strand. No crushed protophloem is recognizable in the primary root.

A pericycle three or four cells in width, opposite the xylem groups and wider over the phloem, forms the periphery of the stele. At places this pericyclic zone of cells is ill-defined and merges into the parenchymatous rays which are at right angles to the length of the xylem groups.

At a level of 2 cm. below the divergence of the cotyledons (the primary root), the Casparian strips on the radial walls of the cells are so small that they are scarcely visible. At a higher level, in the hypocotyl, the Casparian strips are pronounced but are entirely unrecognizable at a level about 1 cm. below the divergence of the cotyledons. In general the endodermal cells are larger than the cells of the stele adjoining them, and are devoid of intercellular spaces.

The cortex of the root is usually less than six or seven cells in width. The cells are spherical with relatively small intercellular spaces. The epidermis of somewhat elongated tabular cells is soon sloughed off. Root hairs are found only rarely. With rapid growth of the root the endodermis often collapses early and the entire cortex sloughs off. No evidence of a periderm was found.

As indicated in figure 2, which is a cross-section 2 mm. higher than figure 1, the axis increases slightly in diameter. Viewed at this level the wedges of primary xylem appear more obtuse and farther apart. In reality the protoxylem points remain in the same position relative to the diameter of the axis, while cells more lateral and more abaxial to them become differentiated and mature into metaxylem.



At the same time a larger number of cells in the center of the axis remain parenchymatous and constitute the pith. At this level (fig. 2; also fig. 17, level 2') and at all succeeding levels the axis is a dissected siphonostele.

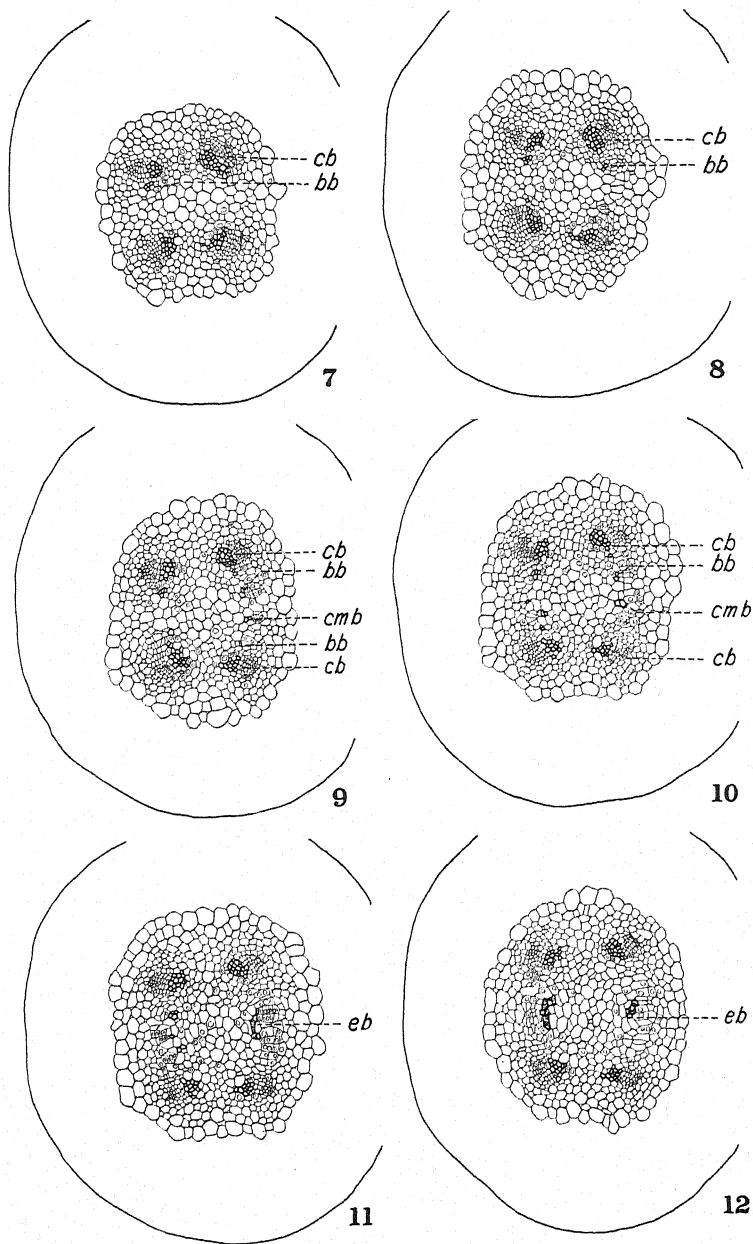
As shown in figure 3 (also fig. 17, level 3'), the metaxylem continues to be differentiated more centrifugally and tangentially higher in the hypocotyl, so that in cross-section each primary xylem group has the shape of an equilateral triangle with the protoxylem point exarch.

About 3.5 mm. below the divergence of the cotyledons (fig. 4), a section of the hypocotyl shows that each of the two exarch primary xylem strands becomes divided tangentially, appearing as a pair of xylem groups. These four strands of primary xylem continue into the cotyledons although they become endarch in arrangement; and each pair constitutes, together with their associated phloem groups, a cotyledonary trace. Casparian strips are best developed at this level. In all succeeding cross-sections the endodermis is doubtfully recognizable by its size, relative position, and the lack of intercellular spaces.

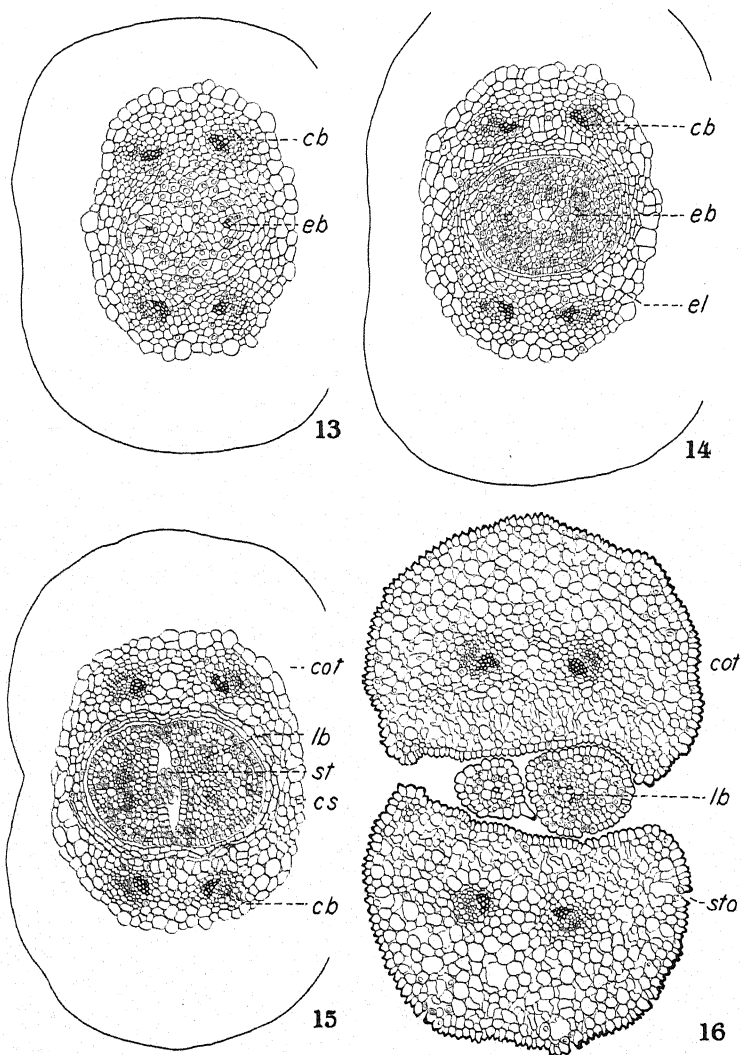
Cross-sections nearer the cotyledons show the following:

1. The metaxylem elements are differentiated in a progressively abaxial direction with reference to the protoxylem. At a level indicated by figure 7 (and fig. 17, level 7') a condition exactly intermediate between exarch and endarch is shown. In succeeding higher levels the centrifugal differentiation continues, so that when the cotyledonary trace enters the cotyledons the bundles are completely endarch.

2. At the level of cotyledon divergence each cotyledonary bundle gives rise to a branch bundle (fig. 7 and following figures). The branch bundles depart adaxially and slightly toward the outside of the axis, so that at a slightly higher level a pair from opposite cotyledons meet. Just before the bundles of each pair anastomose with one another, another bundle composed of a few tracheids is differentiated between them. This new bundle (figs. 9, 10, *cm b*; fig. 17, levels 9', 10'), which is designated as a complementary bundle, arises *de novo* with no connection with other vascular tissue at a lower level. This short bundle anastomoses with the converging



FIGS. 7-12.—Transverse sections of hypocotyl of a seedling 33 days old: Figs. 7, 8, appearance of branch bundles (*bb*) from cotyledonary bundles (*cb*). Figs. 9, 10, appearance of complementary bundle (*cm b*) with no vascular connection at lower end. (In fig. 10 the lower right branch bundle has failed to mature at this level.) Figs. 11, 12, anastomosis of a pair of branch bundles with each other and with complementary bundle to form epicotyledonary bundle (*eb*).  $\times 47$ .



FIGS. 13-16.—Transverse sections of epicotyl (*el*) and cotyledons of a seedling 33 days old: Fig. 13, divergence of abaxial surface of cotyledons (*cb*, cotyledonary bundle; *eb*, epicotyledonary bundle). Fig. 14, divergence of adaxial surface of cotyledons. Fig. 15, transection through tip of axis. Cotyledons (*cot*) have not diverged from each other at this level (*lb*, leaf bundle; *st*, stem tip; *cs*, cotyledonary sheath). Fig. 16, transverse section through cotyledons and tips of first foliage leaves (*sto*, stoma).  $\times 47$ .

pair of branch bundles from opposite cotyledonary bundles to form a single epicotyledonary bundle (figs. 11, 12, *eb*). Each epicotyledonary bundle is therefore connected at its base to three bundles; two

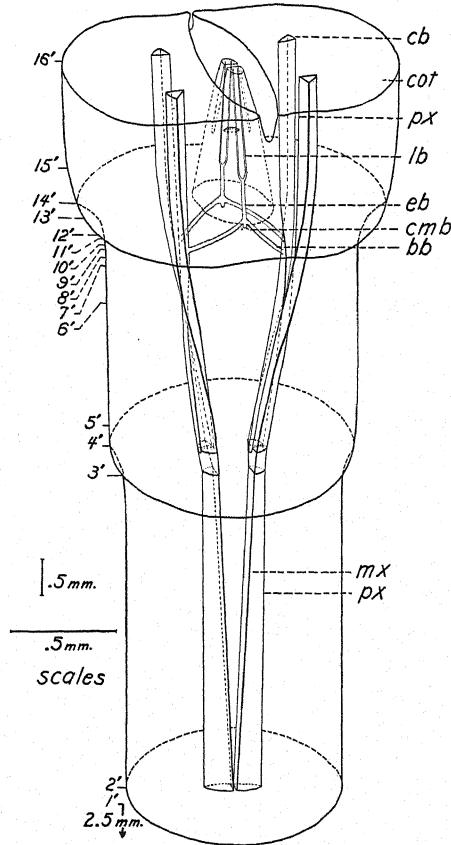


FIG. 17.—Schematic, longitudinal diagram of hypocotyl showing transition from root to cotyledons (drawn to scale with the levels of figs. 1-16 indicated as 1' to 16'). Horizontal and vertical proportions are respectively three and one (*cb*, cotyledonary bundle; *px*, protoxylem; *mx*, metaxylem; *lb*, leaf bundle; *eb*, epicotyledonary bundle; *cm b*, complementary bundle; *bb*, branch bundle).  $\times 47$ .

of these are tangential extensions from opposite cotyledonary bundles, and one is a free bundle, arising *de novo* between these two.

At a later age the epicotyledonary bundle becomes a wide crescen-

tic girdle of vascular tissue resulting from the maturation of additional metaxylem elements. From the two crescentic girdles of vascular tissue at each node (in the first node designated as epicotyledonary bundles) two pairs of bundles arise, while two pairs continue through this girdle into the next internode and depart into the succeeding higher leaves (figs. 18-25).

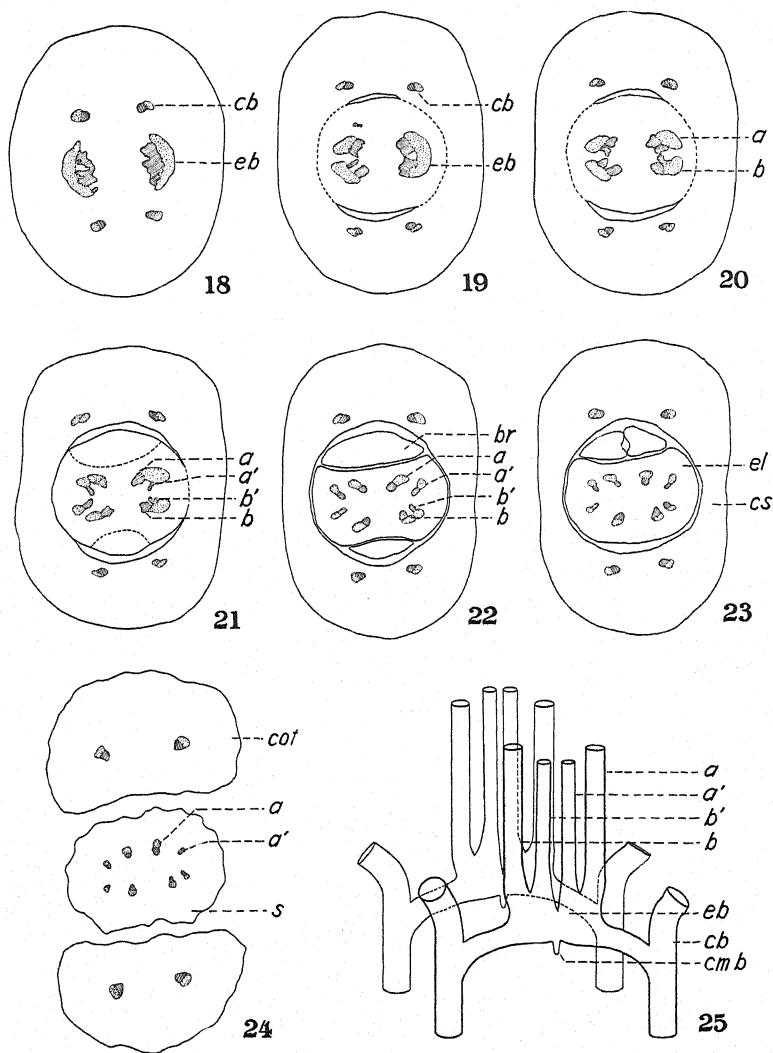
#### COTYLEDONS

The opposite cotyledons as well as the opposite leaves do not diverge from each other for some distance above the node, thus forming a sheath (figs. 14, 15). The flattened side of the semicircular cotyledons (as seen in cross-section) usually is concave, conforming to the curvature of the axis. The epidermis of elongated cells (four to five times as long as broad) is heavily cuticularized. In cross-section the radial and tangential dimensions of the cells are approximately the same. The stomata are sunken, the aperture communicating with the outside being guarded by four to six epidermal cells (7). Between the epidermis and the two vascular bundles of each cotyledon chlorenchymatous cells of various shapes are found. Those near the bundles are compact while those near the periphery have air spaces between them. Under each stoma the air space is relatively large. Near the base of the cotyledon the adaxial subepidermal cells are elongated palisade cells, with their long axis perpendicular to the axis of the foliage organ. In the upper end of the cotyledon (which emerged last from the seed coat) no definite palisade layer is recognizable. In the greenhouse the cotyledons function as photosynthetic organs for more than six months.

The phloem, which in the primary root was in four groups, two lateral to each of the two xylem groups, is differentiated abaxially at successively higher levels, so that in the cotyledons it is completely centrifugal with reference to the xylem. In the cotyledons the transition is complete; thus only the hypocotyl and the cotyledons have been directly involved.

#### EPICOTYL AND STEM

At the age of 33 days only two decussate pairs of leaves have formed. The lower internode of these two shows some provascular



FIGS. 18-25.—Figs. 18-24, transverse sections at level of cotyledon divergence and through epicotyl of a seedling 51 days old. Figs. 18, 19, transverse sections at cotyledonary node. In fig. 19 the adaxial surface of the cotyledons is diverging from the axis and each epicotyledonary bundle (*eb*) is beginning to diverge at right angles to its length (*cb*, cotyledonary bundle). Figs. 20, 21, each epicotyledonary bundle (crescentic girdle) diverges equally to give rise to two bundles, *a* and *b*, which in turn diverge into *a*, *a'*, and *b'*, *b*. Fig. 22, cotyledons diverged completely from epicotyl. Buds in axils of cotyledons have given rise to branches (*br*). Figs. 23, 24, eight bundles in the stem differentiated so that they are nearly equidistant from one another (*el*, epicotyl; *cs*, cotyledonary sheath; *col*, cotyledon; *s*, stem).  $\times 25$ .

Fig. 25.—Schematic diagram of vascular system in epicotyl and stem based on transverse sections shown as figs. 18 to 24 (*a*, *a'*, *b'*, *b*, as in figs. 21 and 22; *cm b*, complementary bundle).  $\times 53$ .

tissue, the upper one none. No cell elongation has taken place. In the lower internode two opposite points, alternate in arrangement with the cotyledons, soon are sufficiently mature to be identified as protoxylem and protophloem elements and are continuous with the epicotyledonary bundle. At a higher level each one of these points branches equally, each pair of bundles constituting a leaf trace. In figure 16 the xylem elements in the first pair of leaves above the cotyledons are more mature than those in figure 15. Growth by cell division and cell enlargement brings the foliar bundles farther apart in the more distal portions of the leaf.

In cotyledons and leaves which are fully grown, the two foliar bundles run nearly parallel to each other and extend to within two or three cells from the epidermis of the tip. The few cells which intervene between the ends of these bundles later mature into short cells with reticulate thickenings, that is, transfusion tissue.

In seedlings which are 51 days old secondary growth has begun in the root and hypocotyl, but practically no cambium has formed in the axis above the cotyledons. Beginning with a section through the cotyledonary node comparable to figure 12, the primary tissues have matured more completely (fig. 18). Instead of the two small protoxylem points (fig. 13), two opposite crescentic girdles of primary xylem subtended by two collateral phloem groups are present. These girdles alternate in arrangement with the cotyledons and are comparable to the cotyledonary plate in other plants. The cotyledonary bundles have diverged at a lower level, as described in connection with the transition.

As the adaxial surface of the cotyledon departs from the axis, each cotyledonary girdle branches equally in the direction of its longest dimension. The direction of this divergence is alternate with that of the cotyledonary trace (fig. 19). Following so closely as to be almost concurrent, each of the four bundles just formed diverges in a plane parallel to the first divergence. Although not all of the eight bundles thus formed depart at the same level, a slightly higher section (fig. 20) shows that eight distinct bundles are present in the axis. In subsequent sections the bundles are differentiated in such a position that they are nearly equidistant from one another. The

two pairs of bundles which departed last are slightly smaller and roughly form the corners of a parallelogram which has its longest dimension at right angles to the cotyledons. These two pairs of bundles constitute the leaf traces for the succeeding node (fig. 25).

Stem tips of *Ephedra pedunculata* and of *E. viridis* were compared to determine the vascular condition of the node. Beginning at a level represented in figure 25, the pair of bundles at the narrow end of the parallelogram diverge into the leaves just before the node is attained. At the node the remaining six bundles are anastomosed into two semicircular girdles, much as the six bundles constitute the girdles (cotyledonary plate) in the first node. The crescentic girdles alternate in position with those of the preceding and succeeding nodes. From the girdles at each node eight bundles arise as described for the cotyledonary node (figs. 18-25). Such origin explains the presence of eight bundles in the stem of *Ephedra* as described by earlier investigators (1, 9, 10, 13, 15, 17). Variations from this number can be explained on the basis of the non-divergence of two pairs of bundles and by the differentiation of a "complementary" bundle between the bundles which form the leaf trace for the succeeding node (17).

*Ephedra viridis* possesses eight distinct bundles in its stem but the lateral differentiation of metaxylem proceeds until a continuous cylinder of xylem is formed, making it appear as though secondary growth began very early in the stem. *Ephedra altissima* (?) resembles *E. pedunculata* in the appearance of the internodal bundles.

### Summary

1. The root-stem transition is described for *Ephedra pedunculata* Englm. and the vascular condition of the stem is compared with that of *E. viridis* Coville and *E. altissima* Desf.
2. The primary xylem in the root of *Ephedra* is diarch, exarch. The two groups of primary phloem alternate with the xylem.
3. The transition occurs in the hypocotyl. Each primary xylem group diverges into two lateral branches both of which continue into the cotyledon. Differentiation and maturation of the xylem elements in each cotyledonary bundle proceed in such a manner that



at successively higher levels the metaxylem becomes constantly more abaxial. The cotyledonary bundles are exarch when they depart into the cotyledons.

4. At the first node the cotyledonary bundles each give rise to a branch bundle. These bundles are differentiated toward each other in pairs. When these converging branch bundles anastomose with each other a third but shorter bundle joins them. The latter bundle arises *de novo*.

5. In an older stem the differentiation of additional metaxylem elements to the three bundles, forming an epicotyledonary bundle, gives rise to a crescentic girdle with its longest axis parallel to the leaves diverging at that node. From the pair of girdles at each node eight bundles arise and continue separately through the succeeding internode. Above the second node each bundle continues for two internodes before it departs into a leaf.

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# STRUCTURAL RESPONSES TO THE PRACTICE OF TOPPING TOBACCO PLANTS: A STUDY OF CELL SIZE, CELL NUMBER, LEAF SIZE, AND VEINAGE OF LEAVES AT DIFFERENT LEVELS ON THE STALK

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(WITH FOUR FIGURES)

## Introduction

In the United States the common practice of producers of practically all but the shade-grown tobaccos is to go through the field a few weeks before the plants are mature and break off the terminal flower stalks. This practice, commonly referred to as topping, hastens the development of axillary branches, which are also removed on subsequent trips through the field. The latter procedure is usually referred to as suckering; but the plants mentioned in this study as topped actually were both topped and suckered. Such practices are in reality little more than the pruning so commonly resorted to for diverse reasons in the production of many plants of economic importance.

Normal growth of the tobacco leaf has already been considered (1), and topping and suckering practices as related to quality and yield have been discussed by others. The general result reported is an increased yield due to the stimulation of growth of the leaves at the upper end of the stalks of topped plants. Does this increase in leaf size mean that the practice of topping stimulates cell division and that the subsequent enlargement of these additional cells is responsible for the increased size, or does the increase come about entirely from the greater enlargement of existing cells? Do all tissues of the leaf necessarily respond to topping in the same manner? The principal purpose of this paper is to answer these questions and to discuss their possible bearing on the problem of the relation of function to differentiation of cells in certain tissues.

### Materials and methods

The plants on which the following data are based were *Nicotiana tabacum* L. varieties Cash, Cuban Shade, and Havana Seed. There were six plants of each variety. The details recorded here are from the Cash variety, and are supported in entirety by less detailed observations on the other two. The seeds were planted in flats in the greenhouse in February and the seedling plants moved into 10 inch clay pots six weeks later. Growth continued over the ensuing 19 weeks, a much longer time than it would have extended under field conditions. Each leaf was allowed to remain on the stalk until fully mature, that is, until it started to turn yellow in color, but was removed before any wilting or shriveling took place. Half of the plants were topped at the 21st leaf on June 22, the other half retaining their flower stalks and flowers. The latter developed seeds (fig. 1).

The dates on which the first seven leaves (the so-called seed-bed leaves) were removed from the plants were not recorded, but the harvest dates of those from the 8th leaf upward are as follows for the untopped plants: 8th and 9th, June 12; 10th and 11th, June 14; 12th, June 20; 13th, June 22; 14th, June 26; 15th, June 28; 16th, July 2; 17th, July 10; 18th, July 13; 19th, 20th, and 21st, July 20. In the topped series, the increase in the duration of the growth period began to be apparent in the 16th leaf, and the dates of harvest from that leaf upward are as follows: 16th, July 13; 17th, July 20; 18th to 21st, August 23.

Samples about 1 cm. in diameter were punched from approximately the middle of the lamina on either side of the midrib of each leaf. These were preserved in formal-acetic-alcohol and were later used for determination of leaf thickness, veinage, cell size, cell number, etc. Leaf area was determined by cutting paper patterns to leaf size, then weighing. The samples used for tissue study were imbedded by the paraffin method and sectioned at a thickness of 12  $\mu$ . They were stained with safranin and fast green.

Determinations of cell size were made with the aid of a Leitz drawing ocular and a planimeter. Veinage determinations were made from the same leaf samples by heating them in a concentrated solution of chloral hydrate until clear, but even after this treatment the

thick upper leaves of the topped plants were difficult to work with. The veinage in a given microscopic field was drawn under low power



FIG. 1.—Effect of topping (photographs taken after leaves for comparative study had been removed): *a, b, c*, plants of Havana Seed, Cuban Shade, and Cash varieties, topped at the 21st leaf above cotyledons; *d, e, f*, same varieties, untopped (normal).

with the aid of a drawing ocular. Magnification and area of field were taken into consideration and the linear measure of veins was then expressed in millimeters per square centimeter of leaf lamina.

## Leaves from bottom to top of stalk in normal and topped plants

### AREA AND THICKNESS OF LEAVES

The gradually increasing size of the several successive leaves above the cotyledons is well understood, and in normal plants with 20 to 25 leaves (meant to include all the leaves ever formed on the plant) this increase ordinarily continues about half-way up the stalk. The next few leaves above this middle level are approximately the same size, and those toward the top are successively smaller. Plants with

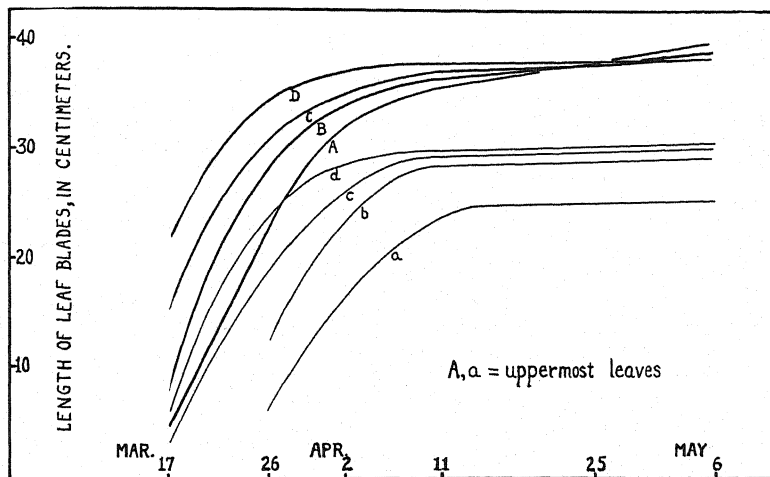


FIG. 2.—Differences in growth of upper four leaves of topped (A-D) and untopped (a-d) plants.

a greater number of leaves have a longer zone in which the leaves are of maximum size. In this experiment the 14th leaf was in the zone of greatest leaf size.

The upper leaves of the topped plants discussed here differ from those ordinarily produced under field conditions, both in their extreme enlargement in proportion to other leaves on the plant and in the fact that they are unusually thick and heavy-bodied to the touch. Topping has little or no effect on the leaves which are mature at the time of the operation, such as those on the lower half of the stalk; but at the time it is done several of the uppermost leaves are only partially developed, and it is these leaves which are stimulated to greater growth (fig. 2). The 17th, 19th, and 20th-21st leaves were chosen

as representative of the upper leaves of the plant. The 17th leaf shows little response to topping; the 19th shows a definite response; and the 20th-21st (averaged and computed as a single leaf) shows a marked response. Taken collectively, these leaves from topped plants average 32 per cent greater in size than corresponding leaves of untopped plants. The average thickness of the upper leaves of topped plants is as follows: 17th, 184  $\mu$ ; 19th, 251  $\mu$ ; 21st, 308  $\mu$ . Corresponding leaves from untopped plants average 188  $\mu$ , 200  $\mu$ , and 188  $\mu$  respectively.

#### SIZE OF PETIOLE AND PETIOLAR BUNDLE

In untopped plants the transectional area of the petioles of successively higher leaves follows the trend of increasing size shown by the laminae of the same leaves, up to the middle of the stalk. Instead of decreasing in size from this level on toward the top, as does the leaf blade, the transectional area of successively higher petioles decreases little or none. In the corresponding petioles of topped plants there is an increase in transectional area which is roughly proportional to the increase in leaf size. Petiolar bundle size maintains a rather constant proportion to that of the petiole in both topped and untopped plants.

#### CELL NUMBER IN VARIOUS TISSUES

While topping does not bring about any observable change in numbers of cells in either epidermal or fundamental tissue, it stimulates secondary activity in the petiole and midrib of the upper leaves (fig. 3). As a consequence there is an appreciable amount of secondary xylem laid down (table I). Fewer lignified primary xylem elements differentiate in petioles of these upper leaves than in corresponding petioles of untopped plants, which suggests that the cambium is stimulated to activity relatively early. The cambium is increasingly active in successively higher leaves.

Cells of the internal pericycle have heavily thickened walls in leaves of both topped and untopped plants. They are easily distinguishable from sieve tubes and companion cells of the internal phloem groups, and the latter are easily distinguishable from other neighboring cells. The sieve tubes and companion cells occurring in

patches (both primary and secondary) were counted as the external phloem. The most careful counts possible on the number of cells of the external phloem parenchyma show no appreciable difference between topped and untopped plants.

It is a noteworthy fact that there are 20 per cent fewer sieve tubes and companion cells in the external phloem in petiolar bundles of the

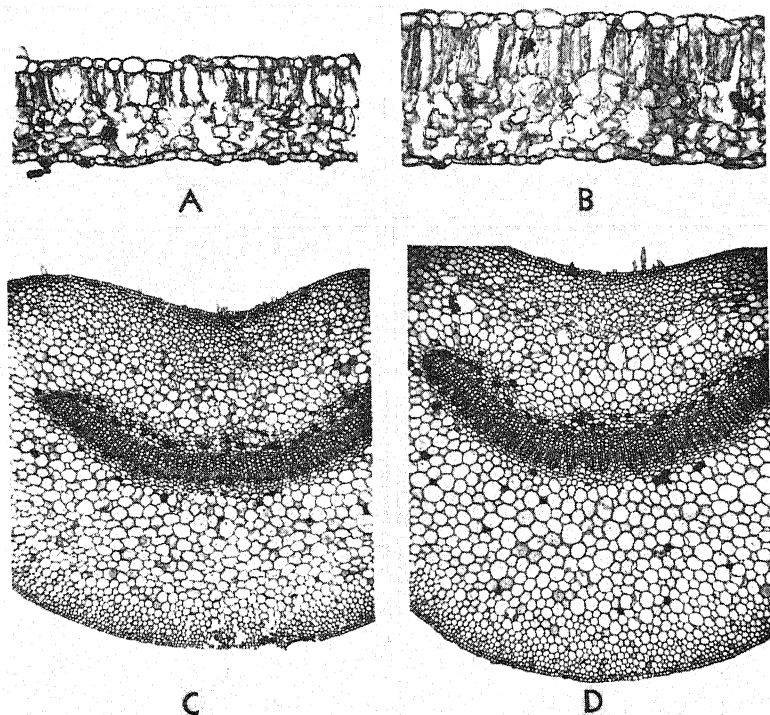


FIG. 3.—Photomicrographs of transections of blade and petiole of 20th leaf of normal and topped plants: *A, C*, untopped (normal) plant; *B, D*, topped plant.

21st leaves of topped plants than in corresponding leaves of untopped plants, a condition quite the opposite to the numbers of lignified xylem elements, despite cambial activity. The numbers of cells in the internal phloem (all primary) remain approximately constant from the 15th leaf upward to the 21st, while in the corresponding leaves of untopped plants the numbers increase sharply (table II).



Observations of ten of the smallest newly differentiated veins (21st leaves, topped plants) showed over 40 per cent fewer phloem parenchyma cells than corresponding veins in untopped plants.

TABLE I

NUMBERS OF PRIMARY AND SECONDARY XYLEM ELEMENTS AND THEIR AVERAGE TRANSECTIONAL AREA IN THE PETIOLAR BUNDLES OF 14TH-21ST LEAVES OF UNTOPPED (NORMAL) AND TOPPED PLANTS

PETIOLE OF LEAF NO.	UNTOPPED (NORMAL)		TOPPED			
	PRIMARY (METAXYLEM ONLY)		PRIMARY (METAXYLEM ONLY)		SECONDARY	
	AVERAGE SIZE IN SQ. MM.	TOTAL NO. OF CELLS	AVERAGE SIZE IN SQ. MM.	TOTAL NO. OF CELLS	AVERAGE SIZE IN SQ. MM.	TOTAL NO. OF CELLS
14.....	0.00210	252	0.00217	246	0.000826	27
15.....	0.00157	276	0.00180	201	0.000744	187
17.....	0.00152	269	0.00131	214	0.000573	293
19.....	0.00140	299	0.00171	223	0.000596	573
21.....	0.00118	352	0.00153			

TABLE II

NUMBERS OF INTERNAL AND EXTERNAL PHLOEM ELEMENTS IN TRANSECTIONS OF PETIOLAR BUNDLES OF THE 17TH, 19TH, AND 21ST LEAVES OF UNTOPPED (NORMAL) AND TOPPED PLANTS

PETIOLE OF LEAF NO.	INTERNAL PHLOEM		EXTERNAL PHLOEM (PRI- MARY AND SECONDARY)	
	UNTOPPED	TOPPED	UNTOPPED	TOPPED
17.....	255	287	382	364
19.....	283	294	360	392
21.....	330	293	411	344

#### CELL SIZE IN VARIOUS TISSUES

The lignified primary xylem elements of the 19th and 21st leaves are larger in topped than in untopped plants (table I), which can be taken to mean only that the response to topping starts early, that is, even before the primary elements (metaxylem) mature. The second-

ary elements are very much smaller than the primary, and they decrease in size more sharply than the latter in the 16th to the 19th leaves.

The increase in size of cells in other tissues in response to topping is roughly proportional to the increase in the size of the leaf as a whole. The upper leaves (17, 19, 21) of topped plants as contrasted with the corresponding leaves of normal plants show the following: upper and lower epidermal cells together average 31 per cent greater in size; palisade parenchyma cells, 31 per cent; fundamental tissue cells in the petiole, 23 per cent greater (table III).

TABLE III

AVERAGE SIZE OF CELLS IN THE 17TH, 19TH, AND 21ST LEAVES OF UNTOPPED (NORMAL) AND TOPPED PLANTS. ALL FIGURES ARE IN SQ. MM., AND REPRESENT AN AVERAGE OF 25 CELLS EACH, EXCEPT FOR THE EPIDERMIS WHERE EACH FIGURE REPRESENTS AN AVERAGE OF APPROXIMATELY 25 CELLS EACH FROM BOTH UPPER AND LOWER

LEAF NO.	EPIDERMIS (UPPER AND LOWER)		PALISADE		"CORTICAL" CELLS OF PETIOLE	
	UNTOPPED	TOPPED	UNTOPPED	TOPPED	UNTOPPED	TOPPED
17.....	0.00258	0.00238	0.00165	0.00175	0.1915	0.1920
19.....	0.00237	0.00318	0.00146	0.00168	0.1635	0.1610
21.....	0.00207	0.00363	0.00189	0.00315	0.1315	0.2475

These results compare with the 32 per cent greater size and the 29 per cent greater thickness of such leaves.

That the lower epidermal cells are consistently smaller than those of the upper epidermis in all the higher leaves is interpreted as meaning that cell division persists longer in this layer.

#### VASCULAR NETWORK AND LEAF SIZE

Table IV illustrates the tendency for successively higher leaves of normal plants to have a greater "density" of veins (greater linear measure per unit area of leaf surface).

In the upper leaves of topped plants there is a tendency for vein-age density to remain about the same from the 15th leaf upward, in spite of the fact that these leaves (17, 19, 21) average 32 per cent

greater in size. This means that additional veins continue to differentiate as the upper leaves of topped plants enlarge, and in sufficient numbers to maintain an almost constant proportion of veinage to leaf surface. Actually the laying down of new veins does not entirely keep pace with leaf enlargement (table IV and fig. 4).

### Relative development of root and shoot in normal and topped plants

Root development of topped plants was markedly greater and the more robust stalks were equally characteristic (fig. 1). Anatomically

TABLE IV  
INCREASED AREA AND DECREASED VEINAGE IN UPPER LEAVES  
OF TOPPED PLANTS AS CONTRASTED WITH CORRESPONDING  
LEAVES OF UNTOPPED (NORMAL) PLANTS

LEAF NO.	AREA IN SQ. CM.			VEINAGE (LINEAR MM. OF VEINS PER SQ. CM. OF LEAF SURFACE)		
	UNTOPPED	TOPPED	PERCENTAGE GREATER SIZE TOPPED LEAF	PERCENTAGE LESS VEIN- AGE IN TOPPED LEAF	UNTOPPED (NORMAL)	TOPPED
14.....	209	.....	.....	.....	.....	.....
15.....	202	184	- 9.8	+13.3	626	709
17.....	184	194	5.4	6.2	696	742
19.....	188	263	40.0	4.4	757	724
21.....	166	254	51.0	9.4	798	723

the roots and stalks of topped plants show greater numbers of heavily walled xylem cells, a characteristic generally associated with plants which are high in carbohydrates.

### Discussion

There seems to be little point here in trying to reconcile the multiplicity of results reported by different investigators, as to height of topping, yield, quality, etc. So much depends upon the nature of the soil on which the crop is grown, the fertilizer used, rainfall and other seasonal influences, as well as the ultimate use to which the tobacco is to be put, that the extent or degree of pruning (topping and

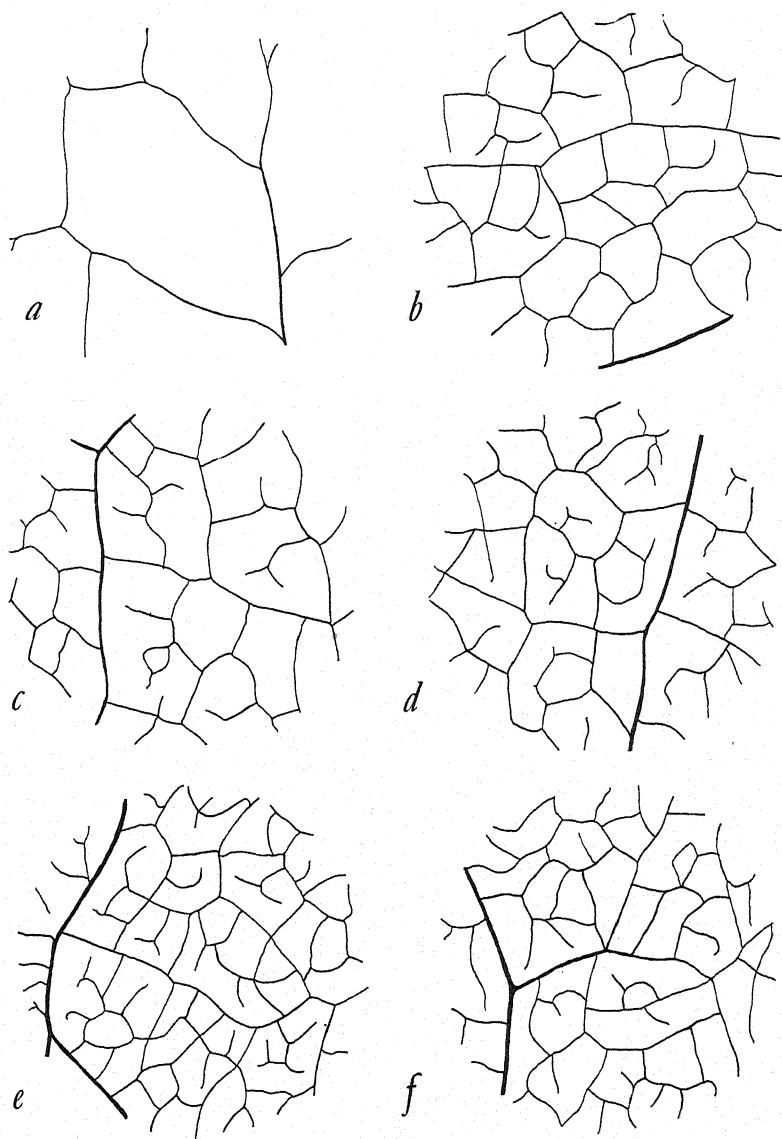


FIG. 4.—Portion of vascular network in cotyledon (*a*) and 4th (*b*), 8th (*c*), 13th (*d*), and 20th leaves above cotyledon. Network of 20th leaf is shown for both untopped (*e*) and topped plants (*f*). Variety Cash.

suckering) which is desirable depends largely upon the specific situation, a fact already well known by agriculturists.

There is, however, general agreement in the literature as to what may be expected from topped tobacco plants as contrasted with untopped plants: (a) They have a greater average leaf surface, depending to some extent on the number of leaves remaining on the stalk (7, 13); (b) the leaves are thicker (7, 13). It is reported that leaf thickness is in direct proportion to the number on the plant, that is, the fewer the thicker (7), or, as expressed by others, the fewer the leaves the coarser the "texture" (3, 4); (c) the growth response to topping is greatest in the upper leaves (4, 7); (d) the duration of the growth period is increased (4, 7), although one investigator later states (5) that topping accelerates the ripening of the leaves; (e) with increased leaf growth there is more extensive development of the root system (4, 5, 7); (f) the physiological explanation of the response to topping and suckering is reported (3) to lie in the removal of many of the users of carbohydrates, making it possible for the young leaves which remain to use most of the material synthesized by them for their own growth.

Such observations quickly resolve themselves into the question of so-called antagonism between vegetative growth and reproduction (10, 11). It has been shown that in certain annual plants with a determinate type of growth, increase in size of plant usually terminates soon after pollination. When the plants are defruited there is a renewal of vegetative development, or if the flowers are removed as rapidly as they open, the plants grow continuously at a uniform rate. The topping and suckering practices in tobacco, similarly, clearly stimulate further vegetative activity (fig. 2). Gross responses to topping substantiate the preceding previously reported investigations, but it is a curious fact that no detailed analysis has been made of the well known increase-in-size response shown by the upper leaves of topped plants.

Other comparable studies, with one exception, tend to bear out the validity of the results reported here. The removal of several of the buds and leaves from a given branch of *Tilia europaea* (6) did not result in excitation of renewed growth in the adult leaves. When done at a sufficiently early period in the development of the leaves,

however, they became abnormally large; such increased size was due to an increase in the number of cells. In another study (18) all apical meristems were removed from *Coleus* and *Phaseolus* plants and only a single leaf was allowed to remain on the stalk. This remaining leaf increased in size owing to an increase in the size of its cells. Comparable results have been reported for the behavior of cotyledons of seedlings (19): By removal of the main shoot as soon as it appeared above the cotyledons, further growth of the cotyledons took place, owing to an increase in the size of certain of their constituent cells. Another study (15) showed that in cotyledons which become hypertrophied early, the epidermal cells increased in size in almost exact proportion to the cotyledon itself; and in those which became hypertrophied later in their development, the increased size was due solely to further cell enlargement. Thus, whether concerned with cotyledons or the leaves which follow, a number of investigators have concluded that increase in leaf size, beyond certain limits, is due to a further increase in the size of already existing cells.

The greater-than-normal growth resulting from topping also finds a parallel in the growth of leaf cuttings of several different kinds of plants: Leaves may live longer when removed from stems and treated as cuttings (8, 9, 14, 18), and if properly cared for they continue to increase in area and thickness. This supplementary growth has been reported as due to an enlargement of existing cells (8), those of the mesophyll, epidermis, and ground parenchyma of the petiole having been observed to increase (9). It has been suggested (14) that the older leaves when left on the plant cease growing because of an inadequate water supply, owing to a diversion to the growing point, etc.

SCHUSTER (17) observed veinage development in leaf cuttings of several species which lived for periods varying from two months to three years. While there was a marked increase in size in the two-months-old cuttings, the veins apparently were merely stretched apart, and veinage ranged from 36 to 87 per cent normal. Those which grew for three years showed additionally differentiated veins, and veinage ranged from 121 of normal in *Hedera helix* to 173 per cent in *Aucuba japonica*. These new veins were reported as arising from dividing spongy parenchyma cells or from "conducting"

parenchyma. SCHUSTER concluded that while the proportion of veinage to leaf surface is apparently an inherent character in normal leaves, the proportion may change if functional demand is changed considerably; and further, that veinage is increased if the "nutrition" is increased. While SCHUSTER's use of "nutrition" is vague, there is a distinct parallel in the upper leaves of topped tobacco plants, where with the increased availability of mineral nutrients and foods there is also an increase in veinage.

It has been noted (16) that "in the great majority of Dicotyledonous plants the increase of xylem within the petiole accompanying increase in size of leaf is brought about by secondary thickening." Whether the laying down of this additional xylem in the upper leaves of topped plants is due to the stimulus of increased demands on the water supply, or whether it is the result of a change in the carbohydrate-nitrogen balance in the leaf, or both, is impossible to say. It is not improbable that a hormone may be involved. High carbohydrate accumulation is often associated with increased secondary thickening, however, and it might reasonably be expected that this same stimulus would promote the differentiation of additional vein network in the enlarging leaf blade.

ALEXANDROV and others (2) have observed that the water conducting system in the petioles of leaves of *Bryonia dioica* is correlated with the size of the leaf, and that the higher the leaf is situated on the stem the greater is the water conducting system leading to it, per unit of leaf surface. The present study bears this out for untopped (normal), but not for topped plants where size of the upper leaves is greater in proportion than is the increase in the water conducting system leading to them (32 and 22.3 per cent respectively).

Whatever the explanation, the evidence is clear that veinage is maintained in almost constant proportion to leaf surface. This suggests that the degree of development of the xylem and the amount of water loss are closely interdependent (12). But while secondary xylem cells differentiate in abundance following cambial activity in the upper petioles of topped plants, there is no further differentiation of secondary phloem in these same petiolar bundles. SCHWARZ (18) has observed an identical situation in leaf cuttings of *Coleus* but has not suggested an explanation. Any conclusion as to why the phloem

fails to increase appreciably, based on the evidence at hand, would be partly conjectural, and it seems sufficient to point out the correlation which exists: Phloem cells differentiate in untopped (normal) plants on which flowers and seeds develop in greater numbers than in topped plants where no flowers or seeds develop, even though the cambium is active only in the topped plants; that is, the absence of developing seeds and the subsequent cessation of diversion of material from leaves to the seeds (diversion of material to the root presumably continues) is accompanied by a cessation of differentiation of phloem cells.

### Summary

1. In the tobacco plant all vegetative growth usually ceases soon after the seeds start to form. If the terminal flower stalk and all axillary branches are removed ("topping" and "suckering") as rapidly as they appear, the upper few leaves on the stalk continue to increase in size for some time. Actually the upper third of the leaves on the stalk had a prolonged growth period in plants which were topped at the 21st leaf; and the 17th, 19th, and 21st leaves of these plants, as contrasted with corresponding leaves of untopped (normal) plants, show 32 per cent greater average area and 29 per cent greater average thickness.

2. This greater than usual growth of upper leaves of topped plants is due to a greater than usual increase in cell size. The palisade and upper and lower epidermal cells average 31 per cent larger. The fundamental tissue of the petiole does not increase in proportion to the tissues of the blade, its cells averaging only 23 per cent larger.

3. The only change in numbers of cells (in leaves 17, 19, and 21) due to topping occurs in the vascular tissue. Cambial activity results in an average of 47 per cent more lignified xylem elements in the petiolar bundle. Differentiation of secondary phloem in the petiolar bundles of these leaves is negligible, despite cambial activity.

4. The following correlations between structure and function in the upper leaves of topped plants seem worth noting: (a) There are fewer secondary phloem cells in the petiolar bundles than in corresponding leaves of untopped plants. This suggests that there is a partial cessation of phloem differentiation associated with the discontinuance of translocation of material out of the leaves to develop-



ing flowers and seeds. (b) The degree of development of the xylem and the amount of water loss are closely interdependent. This is evident from the fact that veinage increases in almost constant proportion to the enlarging leaf blade, and is accompanied by the development of considerable secondary xylem in the petiole.

Appreciation is expressed to Miss EDITH TOMKINS for her assistance in the determinations of cell sizes.

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# EFFECT OF ULTRAVIOLET RADIATION ON GROWTH AND ON THE CALCIUM AND PHOSPHORUS CONTENTS OF PLANTS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 458

HARRIS M. BENEDICT

(WITH PLATE II AND ONE FIGURE)

## Introduction

With the discovery that ultraviolet radiations between 2900 and 3100 Å. caused an increase in the calcium and phosphorus contents of the blood of animals, plant physiologists began investigations to determine whether or not these rays were of importance to plant growth. To date these investigations, which have been so thoroughly reviewed by POPP and BROWN (7) that it is not necessary to discuss the literature here, have not been able to show conclusively that these wave lengths have much effect on the dry weight or on the organic constituents of the plant. With one or two exceptions (BEESKOW 4, and FULLER 10), however, they have neglected the ash elements of the plant.

At the time this investigation was started, no paper had appeared on the effect of ultraviolet radiation between 2900 and 3100 Å. on the calcium and phosphorus contents of plants, and the reported results were so conflicting and open to question that it seemed advisable to investigate this subject further.

For any radiation to affect the object it strikes it must be absorbed by that object; therefore if certain wave lengths are transmitted by a leaf they cannot affect it. If the epidermis of a leaf transmits certain wave lengths which the rest of the leaf absorbs, however, the radiation absorbed will bring about some change in the interior of the leaf. It may be only a rise in temperature, but it may also bring about chemical changes such as in photosynthesis, or it may modify or decompose the constituents of the protoplasm such as enzymes, sugars, amino acids, etc. It is important therefore to study the absorption of the ultraviolet rays by the leaf and to observe any differences that

may occur. In investigations in which glass filters and quartz mercury arcs were used to furnish the different regions of the ultraviolet, the possibility was kept in mind that these glass filters might leak a small amount of the harmful rays shorter than  $2900 \text{ \AA}$ , which would offset any beneficial effect derived from the region between  $2900$  and  $3100 \text{ \AA}$ . On the other hand, in plants which were stimulated under these conditions it was possible that the upper epidermis might filter out these harmful rays, preventing them from reaching the photosynthetic cells directly beneath the epidermis.

From some preliminary experiments to determine the effects of this region of ultraviolet on plant growth, there were indications that after the plants were about four weeks old these radiations had no effect whatever. It seemed possible that this lack of influence might be explained on the grounds that with increasing age the epidermis became more opaque to these wave lengths, finally absorbing all of them, so that they could have no effect on the photosynthetic cells in the interior of the leaf.

As no conclusions as to the capacity of transmission of the leaf epidermis, nor as to the changes in such capacity with age, could be found from previous publications, spectrograms were made of the light transmitted by leaves and their upper epidermises, employing species previously used in ultraviolet experiments.

### Methods

Three compartments 12 feet long, 3 feet deep, and 7 feet high were constructed. Each of these was protected from radiations from any other compartment. The only light other than the lights hung in the compartments came in through small windows in the north side of the room, and this extraneous light was kept from the compartments by black curtains hung across the front of them.

In each compartment were hung two 1000 watt tungsten lamps with reflectors and a Cooper-Hewitt Uviol mercury arc equipped with an aluminum reflector. The mercury arcs were suspended between the tungsten lamps and provided the blue and ultraviolet radiations, the tungsten lamps furnishing the longer wave lengths. No attempt was made to filter out the infra-red radiations present.

The ultraviolet radiations were controlled by the filters described

by WITHROW (9). The arc in compartment I was not filtered, but the intensity was reduced to that of the other compartments by the use of three layers of no. 600 Du Pont cellophane. The radiations in this compartment ranged from 2650 to 7200 Å. plus the infra-red rays. The mercury arc in compartment II was filtered with two layers of no. 600 cellophane soaked in a solution of sodium benzoate. This filtered out all the wave lengths shorter than 2900 Å. The mercury arc in compartment III was filtered with two layers of the same

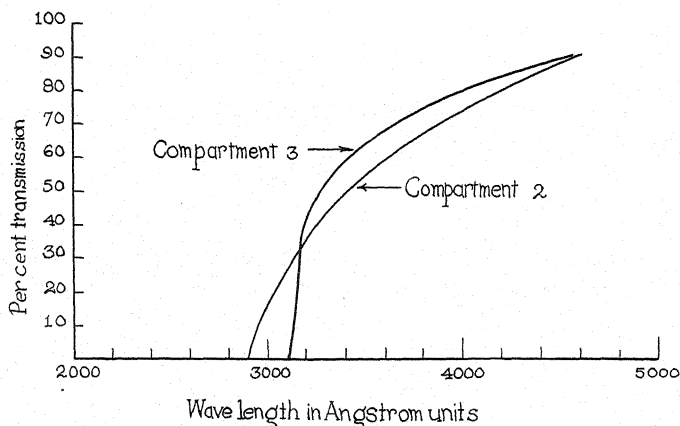


FIG. 1.—Absorption spectra of filters

cellophane soaked in a solution of potassium acid phthalate. This filter absorbed all radiations shorter than 3130 Å.

The absorption curves of these filters are shown in figure 1. As can be seen from the curves, the energy transmitted by the two filters is practically identical, the areas under the two curves being approximately the same. Thus compartments II and III have the same intensity but different wave lengths of ultraviolet radiation. The intensity of the visible radiation in all three compartments was measured with a Macbeth illuminometer and found to be the same, with an intensity equal to 200 foot-candles. The amount of infra-red radiation is not known, but inasmuch as the cellophane filters have little absorption in the infra-red region, it seems safe to assume that the intensity in all the compartments was the same. Recent work by ARTHUR (2) seems to indicate that infra-red rays have little or no

effect on the dry weight of plants. These filters were changed every two weeks and showed no change in transmission in that time. The lights were hung at a distance of 40 inches from the tops of the pots placed in the compartments.

The temperature and humidity of the compartments were not controlled, but records show that the temperatures of the compartments never varied more than  $2^{\circ}$  C. from one another, with a mean around  $25^{\circ}$  C. The humidities of the compartments never varied more than 10 per cent, with an average humidity of 50 per cent.

The soil used was a rich loam. It was mixed thoroughly before using to insure equal soil conditions for all the pots in the three compartments. The soil was sterilized and placed in 8 inch pots, forty of which were placed in each compartment. While the plants were growing, the pots were supplied with an equal amount of water once a day.

The seeds were planted and the lights turned on immediately. The lights were in operation from 6:00 P.M. to 8:00 A.M. daily, during which period the curtains were left open in order to keep the temperature as low as possible.

Three series of plants were grown. The first series was planted November 22, 1931 and harvested one month later on December 22. This series will be referred to as series A. The second series was planted January 14, 1932 and harvested two months later, on March 14. This series will be referred to as series C. The third series was planted April 1, 1932 and harvested about six weeks later on May 19. This, which will be referred to as series B, was killed by escaping ammonia gas, but was harvested immediately; it is not felt that for these determinations the results were affected seriously. Each series can be divided into three sets. Set 1 consists of plants grown in compartment I and receiving ultraviolet radiations as short as  $2650 \text{ \AA}$ .; set 2, of those plants grown in compartment II and receiving wave lengths as short as  $2900 \text{ \AA}$ .; set 3, of those plants grown in compartment III and receiving wave lengths as short as  $3100 \text{ \AA}$ . The upper limit of wave length all the sets received was  $7200 \text{ \AA}$ . plus the infrared rays. The conditions of the three sets of any one series were as nearly alike as possible, with the exception of the wave length of ultraviolet present.

When ready for harvesting the plants were washed from the pots and the entire plants dried at 100° C. for one hour, then at 60° C. for 12 hours in a ventilated dryer. The plants were then weighed and dry weights recorded.

Calcium was determined by the method involving titration with 0.1 N potassium permanganate (3). Phosphorus was determined by the method outlined by ROE, IRISH, and BOYD (8) for blood phosphorus as modified by COCKEFAIR (5) for use in plant analyses.

The experiments with the penetration of ultraviolet were carried out as follows: A hydrogen arc operating at 4400 volts and one-half ampere was used as the source of radiations. The spectrograms were taken with a Hilger E 3 spectrograph.

The leaves were left on the plant while the spectrograms were being taken. They were placed in front of the slit, held firmly in a flat position, and exposed to the radiation for 20 minutes. This length of exposure was used after it had been found that it did not produce much injury to the leaf, but was of ample length to affect the plate.

In exposing the upper epidermis only, the leaf was placed with this epidermis down on a smooth surface and the lower epidermis and the mesophyll carefully scraped away, leaving only the upper epidermis. This was then examined with a hand lens to be sure that no holes were present. The epidermis was then exposed to the radiation in front of the spectrograph for 30 seconds. It is admitted that this is rather severe treatment, and the epidermis under these conditions may not be normal, but the procedure seems less likely to modify the optical properties than would stripping the epidermis from the leaf.

### Results

The results of the determinations of dry weight, calcium, and phosphorus contents of the plants of series A, B, and C are shown in tables I, II, and III respectively. The dry weights are expressed in grams per plant, and the calcium and phosphorus contents as percentage of the dry weight.

In general there is not much difference between the dry weights of the plants of the different sets of series A. In series B and C the dry weights of the plants of set 2 are on the whole larger than those

of sets 1 and 3, with the exception of corn and sunflower in series C where the plants of set 1 have the greatest dry weights.

In all the plants of the three series the calcium contents of those in set 2 are higher than those in set 3, with the plants of set 1 usually

TABLE I

NUMBER OF PLANTS, DRY WEIGHT PER PLANT, CALCIUM AND PHOSPHORUS CONTENTS OF PLANTS OF SERIES A

PLANT	SET 1				SET 2				SET 3			
	NO. OF PLANTS	WEIGHT PER PLANT (GM.)	CALCIUM CONTENT (%)	PHOSPHORUS CONTENT (%)	NO. OF PLANTS	WEIGHT PER PLANT (GM.)	CALCIUM CONTENT (%)	PHOSPHORUS CONTENT (%)	NO. OF PLANTS	WEIGHT PER PLANT (GM.)	CALCIUM CONTENT (%)	PHOSPHORUS CONTENT (%)
Tomato.	30	0.011	3.6	.....	31	0.007	2.6	.....	28	0.005	2.1	.....
Sunflower....	.....	.....	.....	.....	40	0.137	3.3	0.62	40	0.205	2.9	0.67
Corn....	30	0.173	1.5	0.76	28	0.141	2.0	0.70	23	0.172	1.1	0.63
Soy bean	17	0.161	1.8	0.73	49	0.150	2.4	0.62	43	0.158	1.6	0.67
Lupine...	10	0.131	1.7	0.70	29	0.139	1.8	0.77	28	0.141	1.6	0.64

TABLE II

NUMBER OF PLANTS, DRY WEIGHT PER PLANT, CALCIUM AND PHOSPHORUS CONTENTS OF PLANTS OF SERIES B

PLANT	SET 1				SET 2				SET 3			
	NO. OF PLANTS	WEIGHT PER PLANT (GM.)	CALCIUM CONTENT (%)	PHOSPHORUS CONTENT (%)	NO. OF PLANTS	WEIGHT PER PLANT (GM.)	CALCIUM CONTENT (%)	PHOSPHORUS CONTENT (%)	NO. OF PLANTS	WEIGHT PER PLANT (GM.)	CALCIUM CONTENT (%)	PHOSPHORUS CONTENT (%)
Corn....	19	0.516	1.9	0.26	16	0.621	1.5	0.68	22	0.515	1.3	0.49
Tomato.	30	0.011	3.0	0.35	23	0.185	3.5	0.30	23	0.150	3.2	0.58
Nasturtium..	59	0.167	2.4	0.45	50	0.192	2.6	0.47	59	0.166	1.9	0.56
Soy bean	16	0.260	2.0	0.35	28	0.493	2.0	0.54	23	0.460	1.8	0.43
Cucumber...	31	0.100	4.4	0.34	21	0.381	4.2	0.54	21	0.258	2.7	0.38

having a calcium percentage intermediate between those of sets 2 and 3.

The percentage of phosphorus is about the same in all three sets of series A. The plants of sets 2 and 3 of series B are about equal in the phosphorus content, which is somewhat higher than that of set 1; while in series C the plants of set 3 have the greatest phosphorus content.



The results of the studies of the penetration of ultraviolet radiations through the epidermal tissues and the entire leaves are shown in tables IV and V. In all cases the transmission of radiation was

TABLE III

NUMBER OF PLANTS, DRY WEIGHT PER PLANT, CALCIUM AND PHOSPHORUS CONTENTS OF PLANTS OF SERIES C

PLANT	SET 1				SET 2				SET 3			
	NO. OF PLANTS	WEIGHT PER PLANT (GM.)	CALCIUM CONTENT (%)	PHOSPHORUS CONTENT (%)	NO. OF PLANTS	WEIGHT PER PLANT (GM.)	CALCIUM CONTENT (%)	PHOSPHORUS CONTENT (%)	NO. OF PLANTS	WEIGHT PER PLANT (GM.)	CALCIUM CONTENT (%)	PHOSPHORUS CONTENT (%)
Sunflower....	48	0.230	3.5	0.71	50	0.199	4.0	0.68	32	0.168	3.6	0.68
Cucumber....	20	0.279	3.9	0.45	18	0.441	5.0	0.52	22	0.226	3.4	0.66
Tomato....	21	0.115	3.6	0.65	22	0.263	4.1	0.60	28	0.165	3.1	0.68
Corn....	31	0.875	1.5	0.39	32	0.735	1.5	0.51	31	0.859	1.1	0.58
Soy bean	22	0.364	2.0	0.49	20	0.504	2.2	0.33	19	0.455	0.8	0.54
Nasturtium..	48	0.161	3.1	0.76	38	0.228	2.6	0.54	50	0.137	1.9	0.71

TABLE IV

LOWER LIMITS OF TRANSMISSION (Å.) OF LEAVES AND EPIDERMISES

PLANT	LEAF	EPIDERMIS
Corn.....	3750	2350
Morning glory.....	3420	2250
Cucumber.....	3250	2400
Tomato.....	3850	2240
Wax bean.....	3850	2250
Bryophyllum.....		2500
Radish.....	3500	2300
Coleus.....	3800	2600
Sunflower.....	3550	2260
Soy bean.....	3500	2500
Sudan grass.....	3450	2260

continuous down to a certain wave length, with no radiation shorter than that wave length transmitted. Table IV shows in the second column the shortest wave length transmitted by the entire leaf and in the third column the shortest wave length transmitted by the upper epidermises of the leaves.

Table V shows the transmission of leaves and their upper epider-

mises at the ages of two, three, and five weeks. The results show a slight decrease in the transmission of both the leaf and the upper epidermis with increase in age. In no case does the upper epidermis filter out all the wave lengths shorter than 2900 Å. Plate II shows a typical spectrogram of the transmission of various leaves and their epidermises.

TABLE V  
TRANSMISSION (A.) OF LEAF AND EPIDERMIS OF VARIOUS  
PLANTS AT DIFFERENT AGES

PLANT	LEAF			EPIDERMIS		
	AGE IN WEEKS			AGE IN WEEKS		
	2	3	5	2	3	5
Wax bean.....	3250	3450	3500	2300	.....	2310
Corn.....	3520	3740	4050	2250	2400	2380
Lupine.....	4800	.....	4800	2270	.....	2300
Radish.....	3330	3340	3800	2240	2240	2390
Sunflower.....	2900	.....	3550	2230	.....	2200
Cucumber.....	3150	.....	3250	2360	.....	2460
Tomato.....	3800	3850	3950	2220	2240	2240

### Discussion

The chief criticism of the conditions under which the plants in these experiments were grown was the low light intensity. For good growth the intensity in the compartments should have been around 1000 foot-candles instead of 200. Since the intensity in all the compartments was the same, however, the results can be considered as indications of the effect of the wave lengths between 2900 and 3100 Å. on the growth of the plants studied; but because of the low light intensity they can be considered as indications only.

ARTHUR (1) has recently stated that the potassium acid phthalate filter transmits certain wave lengths shorter than 3100 Å. This is true if the filter is not used with a Corex D or Uviol glass backing. If this filter is backed with one of these glasses, however, as it was in these experiments, there is no transmission of wave lengths shorter than 3100 Å.

The results of the dry weight determinations on the plants of series A indicate that any effect the ultraviolet regions between 2900

and 3100 Å. and between 2650 and 2900 Å. might have on the growth of the plants does not become apparent until after the plants are a month old, since the plants of the different sets of this series showed little differences between their dry weights. The plants of sets 1 of series B and C, which were six weeks and two months old respectively, showed the harmful effects of the rays between 2650 and 2900 Å. in their decreased dry weight and in the burned appearance of the leaves which were exposed to these radiations. The plants of these two series also showed the possible benefit of the wave lengths between 2900 and 3100 Å. in the increased dry weight of the plants of set 2 which were exposed to these radiations as compared with that of the plants of set 3 which did not receive these wave lengths. This increase in dry weight was most marked in the case of tomatoes and cucumbers, being as much as 90 per cent in the cucumber plants of series C. In both series B and C there was enough difference between the plants of the different sets to tell them apart readily. The plants of set 2 were the largest, with the exception of corn in series C; those of set 3 were somewhat smaller; those of set 1 were usually of a stunted appearance with their leaves inrolled and looking burnt.

From the results obtained under the conditions of these experiments, there are indications that in general wave lengths between 2900 and 3100 Å. stimulate plant growth, and that wave lengths shorter than 2900 Å. are harmful to plant growth.

The calcium content of the plants exposed to the wave lengths between 2900 and 3100 Å. was increased, both in percentage and in total amount per plant. All the plants of sets 1 and 2 in all three series showed this increase in percentage of calcium. The plants of set 2 usually had a slightly higher percentage than those of set 1. These results seem to indicate that the wave lengths between 2900 and 3100 Å. are most effective in bringing about this increase in calcium content, and that the wave lengths shorter than 2900 Å. exert very little influence, possibly even interfering with the intake of this element, since the percentage of calcium in the plants of set 1 was generally not so great as in the plants of set 2.

This increase in calcium started with the early development of the plants. The plants of series A were only a month old and showed no significant differences in their dry weights, yet the increase in the

percentage of calcium in the plants of sets 1 and 2 was already apparent. There are no indications in the results that the percentage of calcium increases or decreases with age. For a given series the soil of the sets was identical (that is, all the plants of one series were grown in the same soil), but the soil used in series B was not the same as the soil used in series A, and the soil used in series C was not the same as was used in series B. This difference between the soils of the different series may account for the differences noted between the plants of the three series.

The results of the calcium determinations seem to indicate that wave lengths between 2900 and 3100 Å. bring about an increase in the calcium content of the plants, and that wave lengths shorter than 2900 Å. have little effect on the intake of this element.

The results of the phosphorus determinations are peculiar. The plants of set 1 of series A show an increase in phosphorus over the plants of set 3, but in the other two series this set shows on the whole a decrease in percentage from that of set 3. The plants of set 2 show an increase in phosphorus in series A and B, but a rather decided decrease in series C, sunflower and nasturtium being exceptions. These results are so variable, however, that it does not seem safe to make deductions as to the effect of these regions of wave lengths on the phosphorus content of the plants studied.

The results of the study of the transmission of the leaf and upper epidermis with increase in age do show a possible increased absorption of radiations as the age increases, both in the leaf and in the epidermis. In most cases the variations do not exceed the limits of variation between leaves of the same age, with the possible exception of corn. Likewise the increase in absorption of ultraviolet rays by the epidermis with increase in age does not become great enough to filter out all the radiations shorter than 3100 Å.

The results of the penetration studies show that in no case did any wave length shorter than 2900 Å. penetrate through the leaf, and in no case did the upper epidermis filter out all the wave lengths shorter than 2900 Å. ELTINGE (6) reported that in the case of tomato, cucumber, radish, and *Coleus* she obtained stimulation by adding the wave lengths between 2900 and 3100 Å., but no stimulation in the case of morning glory, wax bean, and sunflower. The results presented here show that the epidermis could not have acted

as a protection against short wave lengths leaking through faulty filters in the case of the stimulated plants, and at the same time have failed to act as a protection to the plants that were not stimulated, since the transmission of the epidermis of all the plants was about the same.

The evidence presented by these tests indicates that any results obtained with ultraviolet radiation experiments must always be interpreted on the basis of the penetration of rays shorter than  $2900 \text{ \AA}$ . through the upper epidermis of the leaf, and the absorption of these rays by the underlying tissues if they are present in the radiations.

It will be noted that some leaves absorb more ultraviolet radiation than others. This is in general related to the thickness of the leaves. The thick leaf of *Bryophyllum* transmitted no radiations at all; lupine with a relatively thick leaf absorbed all the ultraviolet, and wax bean with a leaf thinner than the lupine leaf absorbed most of the ultraviolet radiations.

### Summary

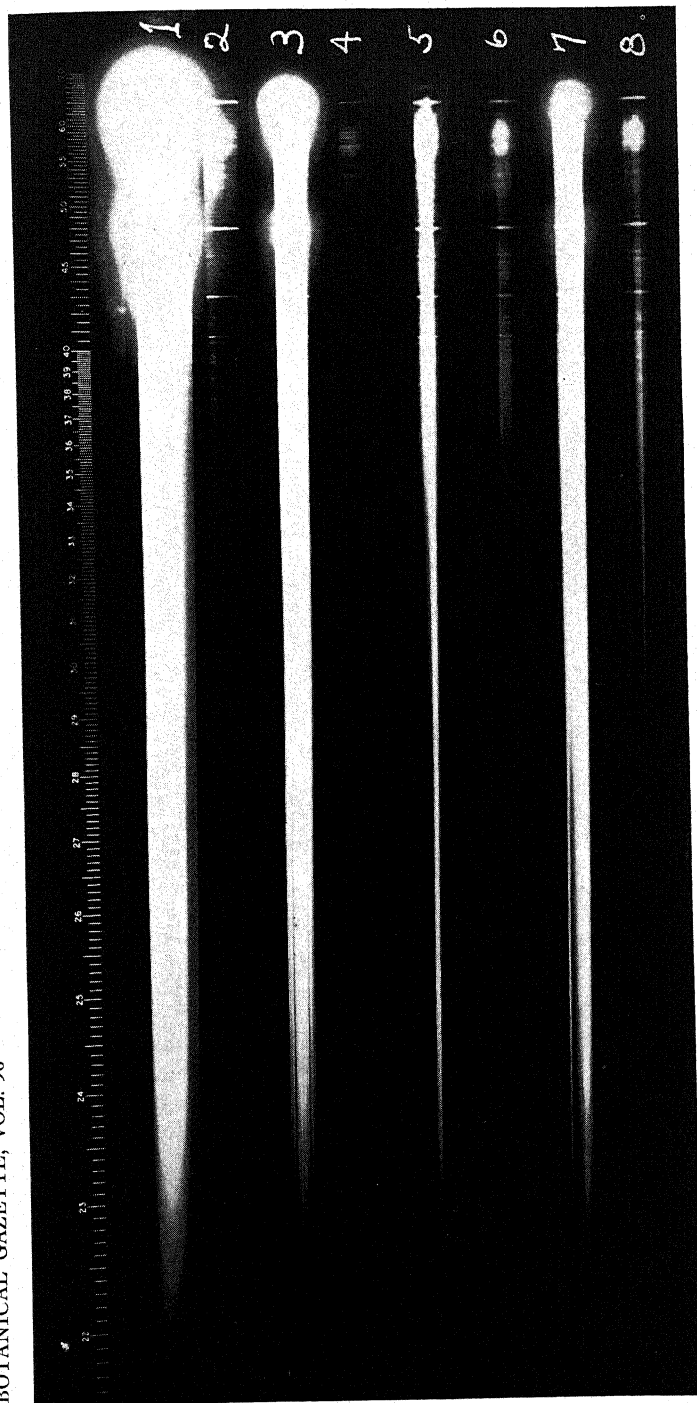
1. Three series of plants were grown and each series was divided into three sets. All the conditions of the three sets of one series were identical with the exception of the range of wave length of ultraviolet radiation received.

2. The amount of growth of the plants was determined by measuring the dry weight. The calcium and phosphorus intakes were also determined for these plants grown in the three different ranges of wave length.

3. The region of ultraviolet between  $2900$  and  $3100 \text{ \AA}$ . apparently caused, under the conditions of these experiments, an increase in the dry weight of all the plants grown with the exception of corn. Plants receiving no wave lengths shorter than  $3100 \text{ \AA}$ . were considered as controls.

4. This same region of ultraviolet ( $2900$ – $3100 \text{ \AA}$ .) apparently caused an increase in percentage of calcium in all of the plants grown. No conclusions could be drawn from the results as to the effect of these wave lengths on the phosphorus content.

5. The presence of wave lengths shorter than  $2900 \text{ \AA}$ . caused a decreased dry weight of the plants but had little effect on the calcium content.



## BENEDICT on ULTRAVIOLET RADIATION

Spectrograms of various leaves and their epidermises. Reading from top to bottom: 1, hydrogen arc; 2, tomato leaf; 3, tomato epidermis; 4, lupine leaf; 5, lupine epidermis; 6, radish leaf; 7, radish epidermis; 8, soy bean epidermis. Some faint lines showing the lowest limits of transmission are lost in reproduction.



6. Transmission studies with spectrograms indicate that the upper epidermis cannot filter out harmful wave lengths of ultraviolet, and that its transmission of ultraviolet radiations does not decrease with age.

The writer wishes to acknowledge his indebtedness to the General Electric Vapor Lamp Company for the loan of the mercury arcs used in these experiments; to the Department of Physics of the University of Chicago and to Dr. H. B. LEMON for the use of the spectrograph. He is also indebted to Dr. C. A. SHULL for advice and criticism throughout the progress of this work.

CENTRAL GREAT PLAINS HORTICULTURAL FIELD STATION  
CHEYENNE, WYOMING

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STUDIES ON PETUNIA. VI  
THE ORIGIN AND DISTRIBUTION OF COLOR IN THE  
ANTHER AND IN THE POLLEN OF PETUNIA<sup>1</sup>

MARGARET C. FERGUSON AND BARBARA HUNT

(WITH PLATE III)

A recent paper (FERGUSON 6) deals with the inheritance of pollen color in diploid petunias. The present paper is concerned especially with the origin, the distribution, and incidentally with the nature, of the pigments in the anther and in the pollen of *Petunia*, including both diploid and polyploid strains.

Here and there in the literature dealing with pigmentation in plants occur scattered references to colored anthers and to color in the pollen, but we have not been able to find reports of any studies based primarily on the origin of color in the anther and in the pollen. However, various writers in the early part of this century were evidently in accord with the view expressed by EAST (4) that "it is well known that pollen color is a tapetal deposit." We are aware of no definite researches on the subject which would give rise to such a generally accepted idea. It may be that the belief has grown out of VAN TIEGHEM'S (17) discussions. He described the growth of the tapetal cells which he said ordinarily take on a yellowish color, and form a complete sheath of yellow cells enveloping the pollen mother cells. He noted that later these cells break down and nourish the young pollen grains, the exine of each grain being yellow in color throughout its entire thickness.

In 1923 MÖBIUS (13) studied the color of anthers and of pollen in 120 species of plants. The list does not include *Petunia*. Eighty per cent of the species investigated had yellow pollen. He states that in about 20 per cent of these the color is due to a yellow oil which is produced by the tapetum and adheres to the pollen grains. He cites KERNER (11) as having found among 520 species, 400 with a

<sup>1</sup> Read before the Botanical Society of America at the Boston meetings, December, 1933.

very thin layer of fatty oil on the outer surface. We do not find, either in the original treatise or in OLIVER'S (12) translation, any evidence that KERNER associated this fatty oil, which he says is usually yellow, sometimes colorless, with pollen color or considered it a factor in the determination of pollen color. There is undoubtedly a very close connection between the yellow pigment of the tapetum and that of the pollen grains; but whether the coloring matter of the latter is secreted by the former, or is synthesized independently in the pollen has not been definitely determined by earlier investigators.

### Methods and observations

#### GENERAL DISCUSSION

When the anthers are fully mature, that is, just prior to their dehiscence, their color may be yellow, varying in different strains from very light to very dark yellow, light gray-blue, light gray-green, bluish purple, or reddish purple. Very rarely we find strains in which the light yellow anthers may contain no pigment. Their apparent color is due to the fact that their walls, composed of white semitransparent cells, inclose yellow pollen. In some strains the anthers are not uniformly colored but present a mosaic. In such cases the background is invariably some shade of yellow on which are streaks or splashes of reddish or bluish purple. The pollen of petunia varies greatly in color. The anther and pollen may have practically the same color, although this very rarely occurs. As a rule they are slightly or markedly different. We have already referred to white anthers carrying yellow pollen. Our large-flowered, deep purple strain, population eight, has, in RIDGWAY (16) terminology, Baryta Yellow anthers and Turtle Green pollen. Our results as to variation of color in these organs are in substantial agreement with MÖBIUS' findings, except that he notes a much smaller range of color in the pollen than do we. This difference is doubtless due to the fact that he worked with species only and our observations are based not only on the species but also on their hybrids.

Both red and blue anthocyanin may occur in the anthers and in the pollen of *Petunia*. Neither color has been found to be present in these organs without the other. Ordinarily they are associated in the same cell, giving tints of purple; but in some instances, as in the

anthers of *P. violacea*, the red and the purple may at times be found in adjacent cells, making a very beautiful mosaic as viewed in section under the microscope. The special "color bodies," to be described later, which are associated with the pollen of polyploid petunias, may contain a mixture of the two anthocyanins, or some may carry blue and others red anthocyanin. Since we so rarely, if ever, find clear blue or clear red in *Petunia*, it would probably more nearly express the actual conditions existing in the plant to say that red anthocyanin may be greatly in excess of the blue in some anther cells and in certain of the "color bodies," and blue in excess of the red in others. The conclusion regarding the presence of two anthocyanins is not based on microscopic observation alone. In comparing the anthers and the pollen, which evidently contain anthocyanin, with the RIDGWAY (16) colors, they are invariably found to correspond to colors which are made up of a mixture of red and of blue pigments. Also the tests for anthocyanin as outlined by BUXTON and DARBISHIRE (3) give very clear evidence of the presence in anther and in pollen of both red and blue anthocyanin.

As reported by ONSLOW (14), BOYLE (2) was the first to use the now classical experiment of fuming white flowers with ammonia. As a result of the fuming his flowers became greenish yellow. FILHOL (8) gave the name xanthogène to the substance responsible for this yellow color and later, 1865, he noted its similarity to luteolin; but not until 1905 was its identity with flavone pigments established by BRIDGOOD (1). When the anthers or the pollen of different species or of cultivated strains of petunias are subjected to ammonia fumes, the results give positive evidence of the presence of at least two flavones, a lighter and a darker yellow, in both of these organs.

#### ANTHER

Since certain of the pigments found in the anthers of *Petunia* are soluble in water, alcohol, and various other liquids, it is not feasible to study their origin and distribution in fixed material. Our study is perforce based on free-hand sections. But with the freshly cut material we also encountered difficulties which could not have been anticipated. When pigment, other than green or yellow, is first observed in the growing anthers, it is invariably a beautiful magenta

red. Because of the ready solubility of this pigment, we were unable to find any altogether satisfactory medium in which to study the freshly cut sections. Rather thin sections of anthers in various stages of development were made from buds of flowers which, when fully open, bear purple anthers. It was found that when these are mounted either in water or in alcohol the magenta pigment disappears almost instantly. Sections were therefore mounted in various other media and immediately sealed with heavy balsam or in a few instances with vaseline.

This pigment is evidently soluble in both cold and hot chloroform, disappearing from the sections as viewed under the microscope in from 6 to 8 minutes. Of the various media used, benzine proved to be by far the best for studying the origin and the distribution of this red anthocyanin, if such it be, with benzene, xylol, and nujol next in order. But in all cases it was essential that the observations be made as promptly as possible after the mounts were prepared, as the magenta color quickly disappears. Whether it passes into solution or is changed chemically we are unable to say. In xylol, nujol, and benzene the bright pigment is lost in from 12 to 15 minutes; but in benzine from 20 to 30 minutes may elapse before any change in the amount or position of this pigment is detected. As the magenta color passes out, in the various media, a bluish pigment, more abundant than the magenta and sometimes spreading over the entire section, appears. As this change takes place, the identity of the cells which earlier carried the magenta red pigment is lost; and, in most of the solvents used, the blue coloration also disappears within an hour. Doubtless the red pigment found in the cells of the growing anthers of *Petunia*, although present in solution in the cell sap, is not an anthocyanin. It combines the solubilities of anthocyanins, carotenoids, and certain flavones. It is possible that it may belong with the red hydrocarbon pigments, which are not carotenoids, mentioned by PALMER (15). We have made no attempt to determine the chemical nature of this magenta pigment; to do so would take us into a field entirely apart from the purpose of this paper.

Just prior to the maturity of the anthers a change occurs both in the color and in the constitution of the bright pigment. The violet or reddish or bluish purple pigment, which during the final steps in

the maturation of the anther replaces in part the red pigment, is doubtless a true anthocyanin. It is soluble in water and in alcohol and insoluble in chloroform, benzene, and similar substances.

The young anthers show no color other than the green which is due to the presence of chloroplastids. But coincident with the formation of the microspore mother cells and the differentiation of the tapetum, a magenta red cell sap appears at four points in the anther as seen in cross-section. Five to seven pigmented cells are grouped at the center of each of the dome-shaped masses of parenchyma tissue which are capped by the four microsporangia (pl. III, fig. 1). When the tapetum has become multinucleated and microsporogenesis is completed, the number of cells containing the magenta red pigment has considerably increased, but this color does not occur outside the cones of sterile tissue (fig. 2). During the period of transition from microspores to pollen grains, the tapetum largely disappears, the endothecium becomes prominent, and the amount of magenta pigment is greatly increased. It has extended much more deeply into the cells of the sterile plates separating the two sporangia on either side of the anther, and has appeared in a very limited number of cells in the inner portion of the anther wall (fig. 3).

Following the maturation of the pollen grains and of the endothecium, the cells connecting the sterile plates and the wall of the anther separate in the line of dehiscence and the two sporangia on either side become confluent. As these changes are taking place, the pigmented cells become very abundant and are scattered throughout the broad plates of sterile tissue (fig. 4). During the entire development of the anther, from the time when pigment other than green or yellow first appears, up to the structural conditions represented in figure 4, no colored cell sap other than the magenta red is present in the anthers of any strain of *Petunia* which we have studied. Just prior to dehiscence, however, a marked change takes place in the pigmentation of the anther, and a violet, a red-purple, or a blue-purple anthocyanin becomes abundant. If the sections are cut at just the right moment, one can see, at points lateral to the connective and on both the anterior and posterior sides of the anther, a violet or a purple anthocyanin extending from the cells with the magenta cell sap at opposite ends of the sterile plates out into the wall of the anther.

The points of origin of the anthocyanin and its line of progression outward are indicated by the arrows in figure 4. In other anthers, the two lines of progressive pigmentation on either side have almost met; and in still others the entire anther wall, with the exception in some cases of the epidermis, is colored with anthocyanin. When the anther is about to dehisce, some of the magenta color ordinarily remains, but in large measure it has lost its vivid color and is replaced by the duller colored anthocyanin. Whether it is actually connected with the origin of the anthocyanin or whether the two simply develop in close proximity we cannot say. The fact that the magenta pigment is at least partially lost, being replaced by the anthocyanin, suggests that the latter originates at the expense of the former.

MÖBIUS (13) found anthocyanin present in the endothecium only in rare instances. When the mature anthers of *Petunia* are uniformly colored over their entire surface, the cells of the endothecium are even more deeply colored than are the other cells of the anther wall.

While as a rule the red and the blue anthocyanins are mixed in the same cell, giving a reddish or bluish purple pigment, we occasionally find, as already mentioned, an exception to this in the anthers of *Petunia violacea*. In this species the anthers may carry a reddish purple anthocyanin, uniform in color throughout all the cells of the wall; but in some instances many cells of the inner layer of the mature anther wall are seen to carry a red pigment, lightly tinged with blue.

In those cases in which the anthocyanin is confined to certain regions of the anther, we have not been able to trace any connection between the color in the cells of the outer wall of the mature anther and the magenta pigment present in the cells of the sterile plate. As the anthers become biloculated, just prior to dehiscence, a bright, reddish purple color appears in scattered cells of the epidermis and here and there in the cells of the inner portion of the wall. We have never found anthocyanin in the cells of the endothecium when the pigment is so distributed in the anther as to form a mosaic (fig. 4).

We have noted earlier that all the cells of the very young anthers contain chlorophyll. Shortly before the origin of the microspores, much of the chlorophyll green is lost and there appears a light yellow pigment rather generally distributed throughout the tissues of the anther. This is a common phenomenon in connection with the loss

of chlorophyll. This yellow pigment gradually becomes more or less condensed, depending on the particular strain of *Petunia*, in the cells of the inner layers of the wall, more especially in the disintegrating cells lining the pollen chambers, and in the pollen grains.

The yellow pigments of the anthers are present in the form of soluble flavones, of chromoplastids, and of an insoluble pigment which may or may not be an insoluble form of a flavone. A portion of the yellow color is soluble in water, and another portion in alcohol; but there remains a yellow pigment which has not passed into solution in any of the solvents with which we have treated it. We shall consider in more detail the solubility of this yellow pigment in our discussion of the pollen grains.

#### POLLEN

In all our cultures of *Petunia*, the mature pollen grains have color. The great variability in color which they present was mentioned earlier. We (6) have recorded to date 38 shades or tints in our various populations breeding true for color, and in their hybrids. These colors fall into the four main groups, blue, yellow, green, and gray. As described in previous papers (5, 6, 7), the typical pollen grains of *Petunia* are ellipsoidal (figs. 5, 6). They are very constant in shape and color in the species and in the true-breeding cultivated strains. In the species and in most other diploid strains, the color of the pollen mass is due to the color of the individual grains. But in polyploid strains, and in diploids with a high degree of heterozygosity, the grains are very irregular in shape (fig. 7 *a*) and, in addition to the color given by the individual grains, there are present very deeply colored structures which we have called color bodies (fig. 7 *b*). These doubtless represent pollen grains which have ceased to grow when only partially developed. As a rule their walls have become excessively thickened and they have acquired, doubtless as the result of an increased acidity in their contents, a very dense solution of anthocyanin. When anthocyanin is not present in the pollen grains, these bodies are bright yellow. The small collapsed pollen grains, evidently dead cells containing some pigment (fig. 8), are not included in our conception of the color bodies.

We agree with MÖBIUS that it is not easy to decide, by use of the

microscope, in which part of the pollen grain the pigment resides. He found only three species in which there was any color in the contents. In a few cases he ascribes, as stated earlier, the color of the pollen to color granules or to a fatty oil adhering to the outer surface of the grains; but in the great majority of the species investigated he found color in the exine only. GERTZ (10) studied the distribution of pigment in over 1500 species and concluded that only very rarely can anthocyanin be said to be connected with the cell membrane. After repeatedly studying pollen grains, in both end and lateral views, after crushing them on the slide, and in certain colorless media which cause their contents to be partially extruded, we have no hesitancy in stating that both exine and contents of the pollen grains of *Petunia* carry pigment. As a rule the exine is more deeply stained with pigment than are the contents. We find no evidence that color in the pollen grains is derived from any special portion of the anther. It seems clear that the conditions conducive to the formation of anthocyanin, or of yellow pigment, in the anther also induce the production of these pigments in the pollen grains. There is no evidence whatsoever that the pollen grains are receptacles of pigments organized and passed on to them by other tissues of the plant.

After extracting the anthocyanin from the anthers and the pollen grains of *Petunia*, they are invariably yellow. This is most strikingly demonstrated in the pollen grains, and leads us to conclude that in the anthers and in the pollen of *Petunia* there is, as it were, a foundational yellow pigment. If yellow anthers and yellow pollen are boiled in water for 15 minutes, the liquid becomes distinctly yellow. A microscopic examination shows that anther and pollen are still yellow. After drawing off the water, adding 95 per cent alcohol and boiling for half an hour, the liquid is again yellow; but anther and pollen are also yellow, the pollen a deeper yellow than the anther. Both the walls of the grains and the crushed-out contents still contain a yellow pigment. Exactly the same results are obtained when 50 per cent hydrochloric acid is used. Sealed mounts were made of yellow anthers and yellow pollen after having boiled them for half an hour each in water, in alcohol, and in hydrochloric acid. After three weeks, the grains and anthers in all three media were still yellow. When these organs are boiled for 10 minutes in ether, in benzene and



similar substances, or in weak sulphuric acid, the liquid is a brighter yellow than after boiling in alcohol, the anther wall is a very light yellow, and both the exine and the partially extruded contents of the pollen grains are distinctly yellow. It is such results as these that have led us to conclude that the yellow pigment in both anthers and pollen of *Petunia* consists not only of carotenoids and soluble flavones, but that a yellow pigment in an insoluble form is also present; and that this insoluble yellow pigment is found in all the pollen grains which we have studied whether or not anthocyanin be present. This insoluble yellow pigment is doubtless identical with, or closely related to, the yellow pigment which has been preserved in the color of spores and of pollen grains during all the eons of time since the coal beds were laid down.

### Summary

1. Throughout this paper, all conditions described or conclusions reached are meant to apply only to those species or strains of *Petunia* which we are growing in our cultures, even though such a limiting phrase may be omitted. At the same time we feel that our observations have probably covered a sufficiently wide range of forms to be of rather general application within the genus.

2. All cells of the very young anthers, except those of the connective, carry chlorophyll.

3. As the chloroplasts are lost, the yellow pigment of the older anthers and of the pollen is initiated.

4. No matter what the color of the mature anther and pollen may be, yellow pigment is always present in them although frequently masked by anthocyanin.

5. The yellow pigment falls into three groups as to solubility, a portion being soluble in water, a second portion soluble in alcohol, and a third portion insoluble in all liquids to which it was subjected.

6. The presence of two flavones is demonstrated.

7. Color, other than green or yellow, first appears in the sterile tissue at the inner side of each of the four microsporangia. It is in solution in the cell sap.

8. This pigment is always a magenta red and is soluble in all the media in which it was mounted. It is evidently not an anthocyanin.

9. True anthocyanin appears just prior to the dehiscence of the

anther. It is first seen in connection with the cells, containing the magenta cell sap, at either end of the sterile plate and from thence progresses outward into the wall.

10. The magenta pigment is evidently closely associated with the origin of the anthocyanin.

11. Both a red and a blue anthocyanin is present.

12. In uniformly colored anthers the anthocyanin may be present in all the cells of the anther with the exception of the connective.

13. With a mosaic distribution of the pigment in the anther, no anthocyanin has been observed in the endothecium.

14. Color is present in both the walls and the contents of the pollen grains.

15. In markedly heterozygous diploids and in polyploids, the color of the pollen is further strengthened by the presence of special color bodies.

16. All the evidence points toward a common origin of pigment in the anthers and in the pollen.

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### EXPLANATION OF PLATE III

#### Color in *Petunia*

All figures have been reduced  $2\frac{1}{4}$  times in reproduction. The magnification of each drawing, as reproduced, is given. The cells containing the magenta pigment or the true anthocyanins are blackened.

FIG. 1.—Cross-section of young anther showing points of origin of magenta cell sap. Tapetum just organized. Microspore mother cells present.  $\times 169$ .

FIG. 2.—Cross-section of one half of a slightly older anther. Tapetum fully organized. Microspore mother cells in early stages of meiosis. Cells with the bright pigment increased in number.  $\times 111$ .

FIG. 3.—Cross-section of one half of a still older anther. Only remnants of tapetum remain. Endothecium fully established; microspores freed from mother cells and mature; pigment much more abundant and more widely distributed.  $\times 78$ .

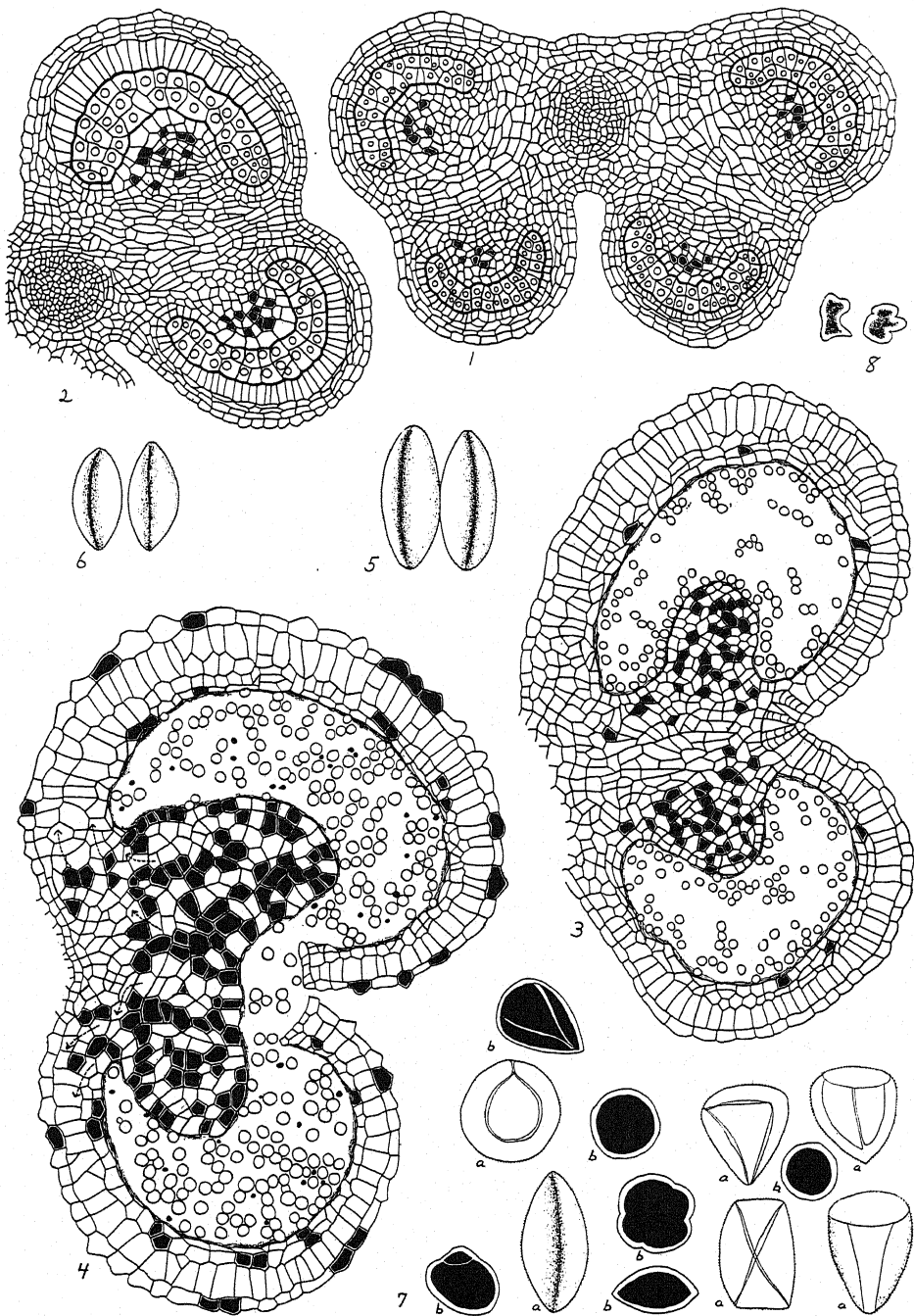
FIG. 4.—Cross-section of one half of an anther just prior to liberation of pollen. This illustrates an anther which has a mosaic color pattern when mature; but the arrows indicate place of origin and course of the anthocyanin when anthers are colored throughout at maturity. Pollen chambers have been formed; pollen is mature.  $\times 78$ .

FIG. 5.—Typical pollen grains of *Petunia* as seen in *P. axillaris*. Both wall and contents are yellow in color.  $\times 320$ .

FIG. 6.—Gray-blue pollen grains of *P. violacea*. Both wall and contents contain anthocyanin.  $\times 320$ .

FIG. 7.—Characteristic pollen of a polyploid: fig. 7 *a*, some of the various shapes of the pollen grains which have pigment in both wall and contents; fig. 7 *b*, a few of the thick walled, densely pigmented color bodies.  $\times 320$ .

FIG. 8.—Small, collapsed, dead pollen grains which are rather densely pigmented but are not considered as special color bodies.  $\times 320$ .



FERGUSON & HUNT on PETUNIA



# STRUCTURE OF MEIOTIC CHROMOSOMES IN MICROSPOROGENESIS OF TRADESCANTIA

KARL SAX AND L. M. HUMPHREY

(WITH PLATE IV)

## Introduction

The coiled structure of meiotic chromosomes has been described in a considerable number of plants, including representatives of both gymnosperms and angiosperms. Although the origin and nature of the coiled structures have been considered by several investigators, there is considerable difference of opinion regarding their interpretation.

KUWADA (4), working with *Tradescantia*, interprets the coiled chromonema as a single coil containing the two chromatids in close association. The two chromatids are assumed to coil in such a manner that for each turn of the spiral there is a twist of the two chromatids about each other in the reverse direction. Such an arrangement of the chromatids would permit their separation without entangling. Such a coiled chromonema could be produced by the contraction of the chromosome pellicle, forcing the two closely associated chromatids into a single coil without rotating or twisting of the chromatids about each other. KUWADA's interpretation has been accepted by BRIDGES (ALEXANDER 1) and by SAX (9).

DARLINGTON (3), basing his argument on theoretical grounds, denies the existence of a single coiled chromonema, and states that "what appears to be a single spiral when considered as a perspective view is undoubtedly two separate spirals when considered as an optical section. . . . The chromosome sometimes seems to be of a zigzag structure, and when apparently a spiral, the spiral may appear to reverse its coiling. This is no doubt due to one of the spirals being taken as a continuation of the other. Each *chromatid* is therefore one spiral, and there is no reason to doubt that this spiral is single and turns in one direction" (pp. 33). DARLINGTON also assumes that the coiling of the chromatids is controlled by the spindle

attachment, and that the direction of the spiral should always reverse at the fiber attachment (pp. 290). CATCHESIDE (2) has accepted this explanation of coiling of chromatids and suggests that such coiling "could be brought about by the revolution of the spindle-fibre attachment constriction about the long axis of the chromosome, the free ends of the chromosome being relatively stationary."

NEBEL (7) has analyzed in considerable detail the direction of coiling of both chromonemata and chromatids in the meiotic chromosomes of *Tradescantia*, and finds that the direction of coiling is more or less at random in the homologous chromonemata on either side of a terminal chiasma, or in the chromatids of the two arms of a single chromosome.

### Chromosome coiling in *Tradescantia*

The present analysis of coiled chromonemata and chromatids was based on material from a *Tradescantia* species related to *T. reflexa* Raf. The species has not yet been described. This species was selected from the many available in Dr. EDGAR ANDERSON's collection, because the anthers are relatively large at the time of meiosis. It is a diploid form with six pairs of chromosomes, and most plants carry one or more pairs of chromosome fragments. A few preparations were also obtained from an  $F_1$  triploid produced by ANDERSON.

Previous investigators have found that various pretreatment methods bring out greater details of chromosome structure. The essential nature of the pretreatment methods commonly used seems to be one of slight dehydration before fixing the material. We have used natural desiccation and immersion in weak alcohol or sugar solutions. The method which gave most consistent results was immersion, after smearing, for 10 seconds in 20 per cent ethyl alcohol (50 cc.) made slightly alkaline by adding a few drops of ammonia water.

After the pretreatment in alcohol, the slide containing the smeared microspore mother cells was covered with fixing solution. Most of the common osmic fixatives gave good results, but a solution containing 2 per cent osmic acid and 1 per cent chromic acid was used most frequently. The preparations were fixed from 3 to 10 minutes and,

after rinsing with 30 per cent alcohol, were placed in 40 or 50 per cent alcohol for half an hour or longer. They were then stained with crystal violet according to Newton's schedule.

The chromosomes of the diploid *Tradescantia* are somewhat heterobrachial with submedian fiber constrictions. At the time of the first meiotic metaphase, both ring and rod bivalents are found. There are often one or two rod bivalents in the metaphase figures. The analysis of coiling of chromonema and chromatids at meiotic metaphase has been confined largely to these rod bivalents, because they can be studied more accurately.

At early metaphase it is perfectly clear that each homologue contains a single coiled chromonema (figs. 1, 2, 5). In most cases it is also clear that the chromonema contains the two chromatids coiled together in close association. The dual nature of the chromonema is shown by the parallel course of the chromatids across the entire width of the coil in many cases. This association is difficult to photograph, but it can be seen in the upper homologue shown in figure 6. In the clearer preparations the chromatids of the coiled chromonema occasionally appear to overlap and form a cross in the upper or lower level of the coil. The single coiled chromonema may persist until early anaphase (figs. 6, 7), although the separation of the coiled chromatids of each homologue is usually complete at this time.

At early metaphase, when the chromatids are associated in a coiled chromonema, the separation of chromatids can be observed at the terminal or subterminal chiasmata (figs. 2, 3, 5). During metaphase the coiled chromatids of each chromonema gradually separate until each homologue consists of two separate coiled chromatids. Various stages in the separation of coiled chromatids are shown in figures 4, 8, and 9, representing bivalents of the diploid form, and in figure 14, picturing two trivalents from the triploid hybrid. In most cases the two chromatids of each homologue are identical in regard to number of coils, direction of coiling, pitch of coils, and any minor irregularity which may be present.

As the chromosomes pass to the poles, each homologue shows clearly two coiled chromatids. In the earlier stages the coiled chromatids are parallel (figs. 10, 11, 12), but at late anaphase they separate, except at the spindle fiber, and form cross-shaped figures (fig.



13). During interphase the chromosomes elongate and tend to straighten out.

At the second meiotic metaphase the two chromatids of each chromosome are rod-shaped and appear much as they do in the usual somatic chromosomes. The chromosomes at this stage are about twice as long as they were at the first meiotic division. At this stage, and especially at early anaphase, it is clear that each chromatid has an internal coiled structure (fig. 15). There are about 20-25 secondary coils in each chromatid, as compared with four or five primary coils found at the meiotic division. These coils appear to be single, and careful study has shown no evidence of split *chromatids* at any stage of meiosis.

We have been unable to determine the direction of coiling of chromonemata or chromatids in all chromosomes of any one microspore mother cell, but it has been possible to get such data for one or several chromosomes in many different cells. An analysis of 165 rod bivalents showed the same direction of coiling on both sides of the terminal chiasma in 91 cases (fig. 1) and a reversal of coiling in 74 cases (fig. 7). The direction of coiling has never been observed to change between the spindle fiber attachment and the distal end of the chromosome, although it is probable that it does so at times where interstitial chiasmata are found. The direction of coiling of the chromonemata may change at the fiber attachment point. In the 165 bivalents studied there were 246 single chromosomes with the chromonemata coiled in the same direction for the entire length of each homologous chromosome, and only 84 chromosomes showed a reversal of coiling at the fiber attachment. Reversal of coiling of the chromonema is shown in the lower homologue in figure 2.

The direction of coiling of the chromatids at anaphase was also studied. Many of these chromosomes were doubtless associated as ring bivalents at metaphase, and the data obtained regarding the direction of coiling on either side of the fiber attachment are perhaps more nearly representative than data obtained from rod bivalents at metaphase. In all cases observed, the two associated (sister) chromatids coiled in the same direction at any given locus. In 90 anaphase chromosomes the direction of coiling was the same throughout the entire length of the chromosomes, and in 38 cases the

coiling was in opposite directions in the two arms of the chromosomes. Reversal of coiling at the fiber attachment can be observed in the anaphase chromosome shown in figure 11.

About 80 per cent of the bivalent chromosomes are associated by terminal chiasmata (or terminal affinity). Most of the subterminal chiasmata observed were so near the distal ends of the chromosomes that it was impossible to obtain data on the direction of coiling on either side of a chiasma. In several cases chiasmata were found near the spindle fiber attachment point, and in one of these bivalents the direction of coiling was reversed at the chiasma.

The pairing of homologous chromosomes by terminal or subterminal chiasmata at the first meiotic metaphase seldom involves the close association of chromatids. As a rule there is a clear space between the distal ends of the chromatids, as shown in figures 2, 3, 5, 8, and 14. This type of association is especially clear in the chromosome fragments. The distance between sister chromatids is about the same as the distance between the ends of non-sister chromatids, and in case of interstitial chiasmata in paired fragments a perfectly symmetrical double cross is often formed. For convenience in discussion, it is assumed that the first meiotic division is reductional, and that no crossing over has occurred.

In both aceto-carmin and permanent smears a hyaline area is often observed around the coiled chromonemata or coiled chromatids. Such a condition suggests that the chromosomes are inclosed by a limiting membrane or pellicle. In cells which have been crushed in smearing, this pellicle is often distended so that there may be considerable space between the cytoplasm and the coiled chromonemata. In such cases the two spindle fiber attachment points, one for each chromatid, are frequently found attached to the pellicle and connected with the coiled chromonema by two fine chromatic strands. These figures seem to show that the fiber attachment points are closely associated with, or definitely attached to, the chromosome pellicle or limiting membrane.

#### Cause and nature of chromosome coiling

Analysis of coiling and chromonemata and chromatids in *Tradescantia* confirms KUWADA's description, and is not in accord with

the interpretation of DARLINGTON and CATCHESIDE. The two chromatids are coiled together in a single chromonema in such a manner that the chromatids can separate without entangling. Such coiling could be induced by compressing the closely associated chromatids within the limiting pellicle in such a way that the chromatids are not permitted to rotate. This type of coiling can be simulated by compressing two closely associated flexible wires in a glass tube while the ends of the wires are not permitted to rotate. The chromatids are undoubtedly somewhat elastic and flexible, as shown by their behavior at division (SAX 9). They may be prevented from rotating within the pellicle by the association between the fiber attachment points and the pellicle. Inhibition of rotation at the distal ends of the chromosomes may also be affected to some extent by the terminal or subterminal chiasmata. The coiling produced in this way would produce the configurations observed. The chromatids, when coiled together at early metaphase, would appear to form a cross in certain chromonematic coils; the coiled chromatids can separate without entangling; and the sister chromatids have the same number of coils, the same direction of coiling at a given locus, and appear identical in any minor irregularities.

The coiling observed in the chromosomes at the second meiotic division is undoubtedly a secondary coiling which exists within the primary coils at the first meiotic division, as described by KUWADA (5). At the second division the primary coiling is absent and only the secondary coiling remains. The coiled chromatids at the first meiotic division are only about half as long as the chromosomes at the second division, but the actual length of the chromatid is about three times the length of the primary coil, so that there must be some contraction of the chromatid, between the first and second meiotic division, by an increase or contraction in secondary coiling. Such secondary coiling may account for the straightening out of the primary coil during the first meiotic division described in *Secale* (9), and chromosome contraction with no primary coils in the meiotic chromosomes of various *Orthoptera* species. BRIDGES' suggestion that coiling is a mechanism essential for the preservation of the linear order of the genes during contraction of the chromosome is in accord with cytological observations if secondary coiling is involved.

The direction of coiling of chromonema and chromatids seems to

be at random for the two homologous chromosomes of each bivalent. In the bivalents described by NEBEL (7) the direction of coiling was approximately at random for paired homologues. In our analysis of rod bivalents, 91 showed the same direction of coiling through the chiasma, and 74 showed a reversal of coiling at the chiasma. This means that when the chromosomes were paired side by side in the prophase stages, there was a slight excess of coiling in different directions, but the differences observed are of doubtful statistical significance.

The direction of coiling on either side of the spindle attachment in the meiotic homologues does not seem to be at random. NEBEL found 83 chromosomes with the direction of coiling unchanged in the two arms of the chromosomes, and 61 chromosomes with a reversal of coiling at the fiber. In our analysis of 128 anaphase chromosomes the direction of coiling of chromatids was the same in 90 chromosomes and was reversed at the fiber in 38 chromosomes. There seems to be a strong tendency for the coiling to be in the same direction at all loci in a meiotic chromosome. The reversal of coiling at the fiber might be expected if the fiber attachment point were a weak place in the chromatid. Such a point of weakness would break the continuity of stress imposed by compression or contraction of the chromatid.

In the case of interstitial chiasmata we should also expect more or less independence of coiling on either side of a chiasma.

NEBEL has observed anaphase chromosomes where the two chromatids on the same side of the fiber coiled in opposite directions, and we have observed one metaphase figure which would give such a segregation. NEBEL is inclined to interpret such figures as products of crossing over according to JANSSEN's theory. It is perfectly obvious that such configurations would be expected with either of the two current theories of chiasma formation.

The usual absence of any intimate association of homologous chromatids at the terminal and subterminal chiasmata seems to invalidate the theory of terminal affinity proposed by O'MARA (8) and by DARLINGTON (3). The homologous chromosomes seem to be associated by a union of the chromosome pellicles, and not by any unusual attraction or bond between the two terminal chromomeres.

According to NEBEL, each of the four coiled chromatids is split so that the bivalent chromosome at metaphase is really eight-parted. We have found no evidence that the chromatids are split, either at the first or second meiotic division. The anaphase chromosomes at the second meiotic division appear to contain a single coiled chromatic thread.

### Summary

1. The chromatids of *Tradescantia* chromosomes are coiled together in a single coiled chromonema at early metaphase of the first meiotic division. At late metaphase the two coiled chromatids in each homologue separate, forming a bivalent with four separate coiled chromatids. The primary coils disappear during interphase, and at the second meiotic division only the secondary coils are found.

2. The primary coils of meiotic chromosomes are assumed to be caused by the contraction or compression of the two flexible chromatids within the chromosome pellicle without rotation of the chromatids.

3. The direction of the primary coils is apparently at random for the homologous chromosomes of each bivalent. Within each homologue the direction of coiling may change at the fiber constriction, but there is a tendency for the coiling to be in the same direction on both sides of the fiber. The direction of coiling may also change at an interstitial chiasma.

4. We have found no evidence that the meiotic chromatids are split in preparation for the division in the microspore.

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## EXPLANATION OF PLATE IV

FIGS. 1-15.—Meiotic chromosomes from permanent smear preparations of microspore mother cells of *Tradescantia*.  $\times 2000$ .

FIG. 1.—Rod bivalent with single coiled chromonemata.

FIG. 2.—Bivalent with subterminal chiasma. Direction of coiling of chromonema is reversed at fiber attachment of lower homologue.

FIG. 3.—Chromatids separating.

FIG. 4.—Separation of coiled chromatids in each homologue.

FIG. 5.—Rod bivalent showing nature of terminal association of homologous chromatids.

FIG. 6.—Early anaphase stage with single coiled chromonemata. Dual nature of chromonema can be seen in the upper homologue.

FIG. 7.—Partial opening out of coiled chromatids at anaphase.

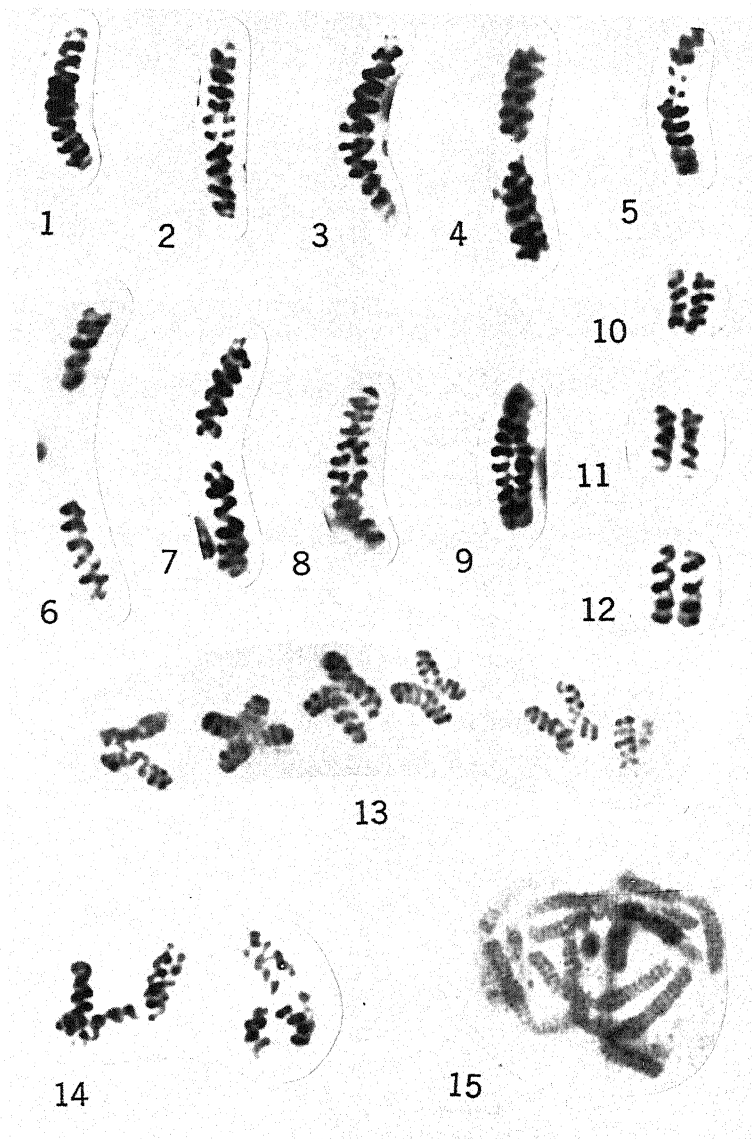
FIGS. 8, 9.—Separation of coiled chromatids at metaphase.

FIGS. 10-12.—Anaphase chromosomes showing direction of coiling of sister chromatids.

FIG. 13.—Late anaphase chromosomes showing characteristic configurations of the six chromosomes.

FIG. 14.—Types of trivalents found in a triploid hybrid. Chromatids showing various degrees of separation.

FIG. 15.—Chromosomes of second meiotic division. Primary coils lost and each original chromatid shows secondary internal coiling.



SAX & HUMPHREY on TRADESCANTIA





# SCHILDERIA ADAMANICA: A NEW FOSSIL WOOD FROM THE PETRIFIED FORESTS OF ARIZONA

L. H. DAUGHERTY

(WITH PLATE V)

During the fall of 1932, Mr. J. A. SCHILDER gave the writer a specimen of wood from the petrified forests of Arizona. Slides were made and it soon became evident that it represents a new wood from the Triassic, and a type of structure not previously reported either in living or fossil plants, justifying the establishment of a new genus. Upon the advice of Dr. I. W. BAILEY, who has examined the slides, it has been decided that, since the specimen shows only the secondary xylem, no suggestions may profitably be made concerning its relationship with other plants until evidence is obtained from additional material showing primary xylem, pith, or leaf traces.

The specimen has been replaced by chalcedony and its petrification is similar to that ordinarily found in the Arizona fossils. Figure 1 (pl. V) indicates the size of the specimen and shows the conspicuous multiseriate xylem rays.

## *Schilderia adamanica* gen. et sp. nov.

TRANSVERSE SECTION (fig. 4).—Growth rings present, distinct, inconspicuous, occasionally doubled and averaging 1.5 mm. in width; variation in width from less than 0.4 mm. to more than 3 mm. Growth rings terminated by from one to five rows of flattened tracheids and a small amount of xylem parenchyma; cells of parenchyma solitary, in groups of two to four or occasionally forming a tangential row. In addition to the terminal parenchyma, some diffuse parenchyma can be found, but owing to the poor preservation it is rather difficult to demonstrate.

The large multiseriate xylem rays (medullary rays) form the most characteristic elements of the transverse section. These are of the "herring-bone" type found in the living genus *Ephedra* and in a few living genera of dicotyledons. They are 1–3 mm. apart, and two or three narrow uniseriate rays may usually be seen between them.

The tracheids appear large, squarish, heavy walled, many over  $50\ \mu$  in tangential diameter, and with walls approximately  $9\ \mu$  in thickness. It is possible, however, that the wall thickness has been slightly increased during petrification.

RADIAL SECTION (figs. 2, 3, 5, 6).—In this and the tangential section the tracheids prove to be of great length and show both pointed and blunt ends. The bordered pits on the radial walls are commonly localized in certain portions of the tracheid and are uniseriate or biseriate. The pits measure approximately  $14\ \mu$  in height and  $18\ \mu$  in width. In the uniseriate condition they are slightly flattened above and below by mutual pressure of the neighboring pits; in the biseriate condition their margins are angled, and the pits of the two rows alternate as is common in the pitting of the araucarian type (figs. 5, 6). The orifice of the pits ranges from almost circular to slitlike, and from a horizontal to an oblique position. When the oblique and slitlike orifice is present, it regularly forms a distinct cross with the orifice on the opposite side of the bordered pit. Bars resembling trabeculae are found extending across the lumina of the tracheids in a radial direction; it is possible that these may represent the hyphae of a member of the wood-destroying fungi.

The multiseriate rays (fig. 3) are composed of a combination of parenchyma cells and tracheids that bend into the ray and unite with it. The tracheids joining with the ray apparently undergo septation and form cells similar to xylem ray tracheids, as the pitting remains similar to that found on the ordinary upright tracheids. The parenchyma ray cells appear to have either pitted or smooth walls where the preservation is good and the minute structure can be seen, but this fact cannot be established with certainty. Many of the details of the multiseriate ray cannot be worked out satisfactorily with the available material, but the fact remains that it is a composite structure, organized only partially from radial parenchyma and consisting largely of longitudinal tracheids that enter the ray.

The uniseriate rays are from one to twelve cells in height, composed of thin walled cells, and usually homogeneous. Heterogeneous rays with certain cells of the ray greater in height than in radial length may be found; the tall ray cells may be marginal, in the central portion of the ray, or make up the entire ray in case it is only

one or two cells in height. The cells of the central portion of the ray are approximately  $30\ \mu$  in height and  $140\ \mu$  in radial length. The lateral walls appear to have from two to six half-bordered pits in each crossing-field; at least some of the well preserved pits are half-bordered and the lateral walls show numerous circular openings believed to be remnants of pits.

TANGENTIAL SECTION (fig. 7).—The tracheids show tangential pits of two sizes, the larger approximately the same size as those on the radial walls, and the smaller about half that size. The pits are circular to oblong in shape and those examined had a slitlike orifice. Spirals may in some cases be seen on the walls of the tracheids, but these are probably the remnants of spiral striations rather than spiral thickenings.

The xylem parenchyma cells are  $60$ – $180\ \mu$  in length, the larger with simple pits on the end walls similar to those found in *Taxodium* and *Sequoia*.

The multiseriate rays show an approximate height of from  $5$  to  $10\ \text{mm.}$  and a width of  $0.3\ \text{mm.}$  The cells appear very irregular in shape in the tangential section, on account of their odd arrangement in the ray. The uniseriate rays are inconspicuous in the tangential sections examined. This is due to the fact that they are rather narrow in tangential width. The tangential width of those measured is approximately  $10\ \mu$ .

It is evident from the description of *Schilderia adamanica* that its wood is of a different type from that of the other two fossil species from this locality. *Araucarioxylon arizonicum* was described by KNOWLTON (1) in 1888 and *Woodworthia arizonica* by JEFFREY (2) in 1910; since that time no new woods have been reported from the Triassic forests of Arizona. It is possible that a careful search of this locality will not only give valuable information in regard to the three species now known, but may result in the discovery of additional new elements of this flora.

The writer expresses his thanks to Dr. I. W. BAILEY for his helpful advice, and for the use of slides during the course of this work.

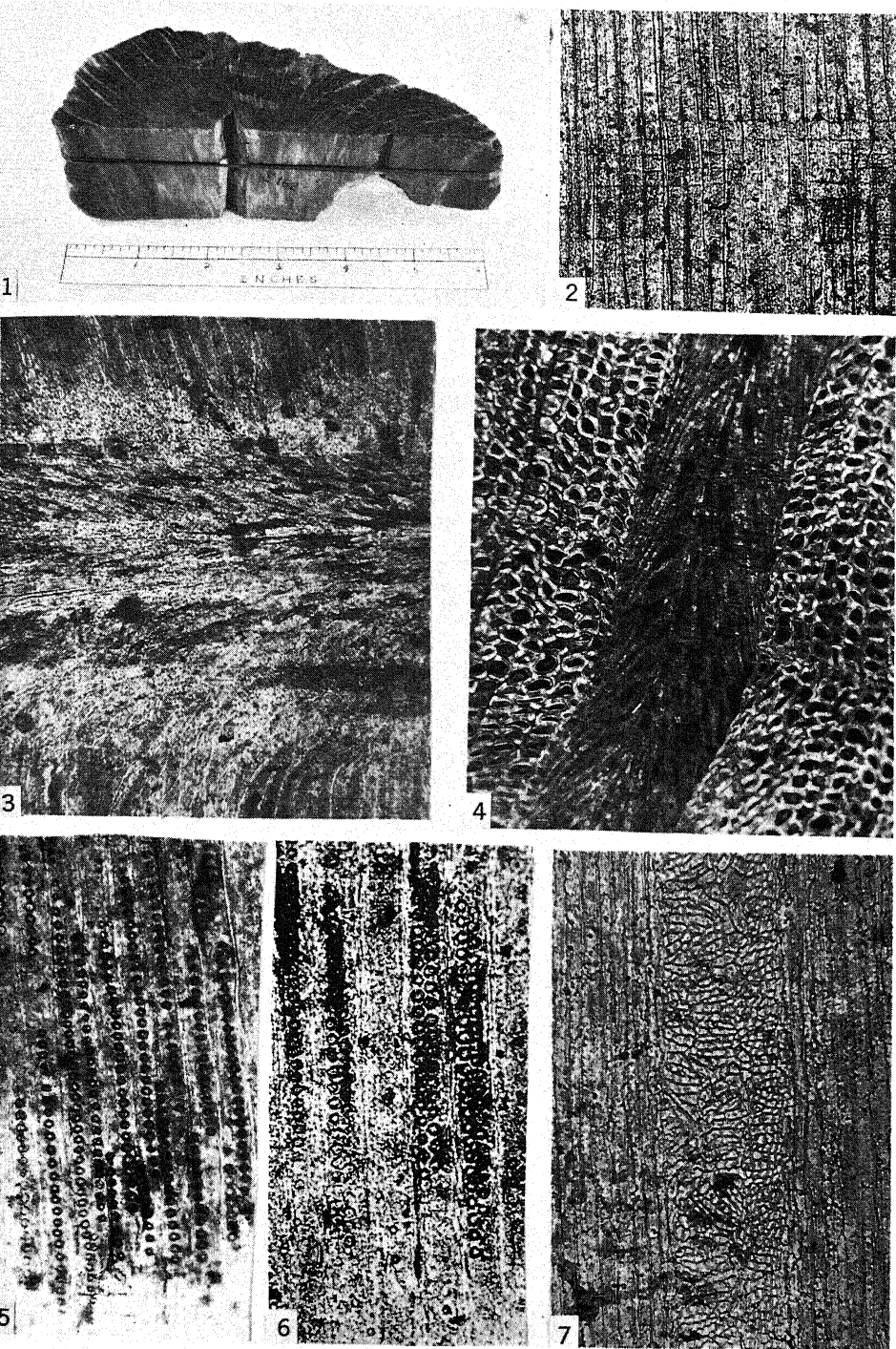
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## EXPLANATION OF PLATE V

- FIG. 1.—Type specimen showing conspicuous multiseriate rays.  
FIG. 2.—Radial section showing uniseriate ray;  $\times 80$ .  
FIG. 3.—Radial section showing multiseriate ray;  $\times 80$ .  
FIG. 4.—Transverse section showing multiseriate and uniseriate rays;  $\times 80$ .  
FIG. 5.—Radial section showing uniseriate type of pitting;  $\times 80$ .  
FIG. 6.—Radial section showing biseriate type of pitting;  $\times 95$ .  
FIG. 7.—Tangential section showing portion of a multiseriate ray;  $\times 80$ .



DAUGHERTY on NEW FOSSIL WOOD



# ANATOMY OF AERIAL ROOTS OF VITIS ROTUNDIFOLIA

LEWIS M. TURNER

(WITH FIVE FIGURES)

## Introduction

BAILEY (1), HEDRICK (4), and SMALL (6) have noted that aerial roots are often present on *Vitis rotundifolia*. They are not so long as, but they otherwise resemble, those of an unnamed, tropical conservatory species as described by MOORE (5). Whereas many die at the end of the first season, a few enter the soil and continue growth, the subterranean part resembling regular soil roots and the aerial part the canes, of course with the absence of nodes, shoots, and so forth. Lateral root development on the aerial part is common the first year, with two, three, or four such arising at a level.

The roots originate on horizontal or oblique canes, usually on the lower side, and their point of origin may be at, near, or remote from the nodes. Since they arise most commonly on canes 1 inch or more in diameter, it seems reasonable to conclude that the phloem parenchyma is their histological source, the pericycle presumably having been lost through periderm formation by the phloem. Since, however, it was not found practical to make a thorough investigation of this problem, the foregoing conclusion is tentative.

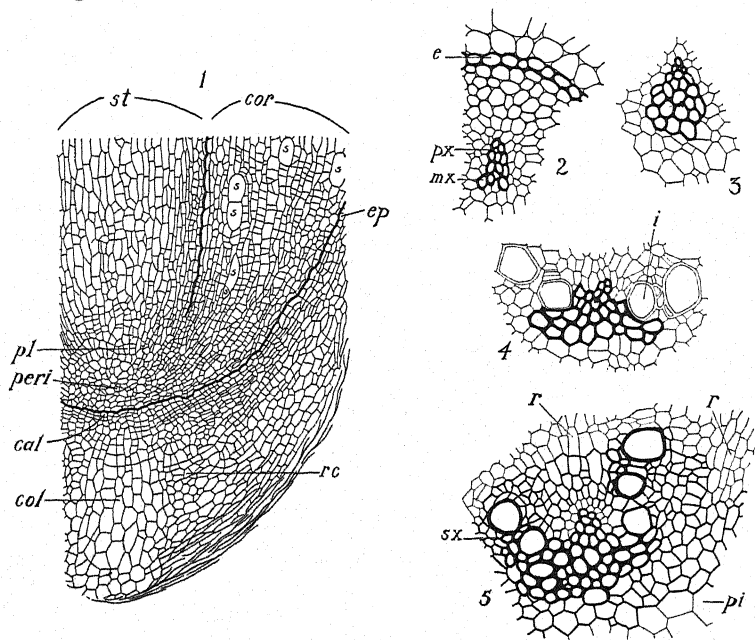
## Investigation

### PRIMARY GROWTH

Three histogens, calyptragen, periblem, and plerome, are distinguishable with difficulty in the apical region of the root. Resembling type II, of the various modes of root cap formation (2), and the root tip of the sweet potato (3), the calyptragen divides early to form the epidermis, the latter persisting as an undivided layer throughout its existence. Except in one respect the performance of the meristems is normal, and is as follows: In the apical center of the conical root cap is a cylindrically shaped area of cells (fig. 1).



This is the "columella" described by NEMEC (HABERLANDT 2) of supposed statolithic function. Its cells are cylindrically shaped with their long axes paralleling the long axis of the root and apposed to the long axes of the adjacent cells of the root cap. In conformity with the statolithic theory it was observed that these cells contain starch grains which are most commonly in the terminal position.



FIGS. 1-5.—Fig. 1, median longitudinal section of apical region of root; figs. 2-5, progressive development of xylem, transverse sections (*cal*, calyptragen; *col*, columella; *cor*, cortex; *e*, endodermis; *ep*, epidermis; *i*, immature secondary xylem; *mx*, metaxylem; *peri*, periblem; *pi*, pith; *pl*, plerome; *px*, protoxylem; *r*, ray; *rc*, root cap; *st*, stele; *sx*, secondary xylem).

The cortex is finally 15 to 25 cells in thickness. Its cells are elongated, with prominent intercellular spaces, and early two or more of its outer layers become collenchyma. This impervious layer would seemingly preclude the possibility of water absorption and in fact raise a serious doubt as to any absorption performance by the aerial part of these roots. The epidermis is lost very early, no stomata are formed, nor were the "pneumathodes" as of orchid roots (2) observed. Storage of crystals and stainable materials in the cortical

cells occurs early in the ontogeny of the root. "Crystal sacs" are visibly differentiated a short distance from the apex, large raphides are formed, are subsequently dissolved, and the cells that contained them collapse.

The endodermis is early distinguishable by the presence of stainable materials in the protoplasm of its cells. As well as could be determined typical Casparian bands are uncommonly found in the irregularly shaped endodermal cells. As a rule there occurs an uneven or interrupted secondary thickening of the walls of these cells. Storage is common, accumulation of materials taking place concurrently with wall thickening, so that by the time the cambium is differentiated the cells are opaque with only moderate staining. Accessory thickening of, and storage in, one or two adjacent cortical layers as occurs in *Taxus*, Cupressineae, *Viburnum*, and some Pomaceae (2) is commonly found.

The primary stele has six, seven, or eight protoxylem points. They are laterally separated by an equal number of primary phloem areas and fundamental parenchyma, and internally by a wide area or central core of pith parenchyma. The pericycle, distinguishable only by its proximity to the endodermis, is separated from the protoxylem points by four or five layers of parenchymatous cells (fig. 2). The centripetally developing protoxylem consists of six to eight spirally or annularly thickened elements. Subsequently several scalariform, and later a few reticulated metaxylem elements, with oblique end walls, develop centripetally and tangentially.

In general the direction of differentiation from primary to secondary xylem is at first centripetal, then tangential (from both corners of the protoxylem "triangle"), and finally centrifugal (figs. 2-5). A large pith core remains in the axis.

The first phloem elements are elongated parenchymatous cells with oblique end walls. Later elements, presumably metaphloem, are tubes of larger diameter with oblique end walls having small scattered, secondarily thickened areas.

#### SECONDARY GROWTH

Cambium is differentiated in the usual manner and position, forming at first an undulating line (transverse view) between primary

phloem and xylem. Two or three layers of the fundamental parenchyma opposite and tangential to the protoxylem points remain undivided, and either become the first and innermost cells of the primary rays or remain parenchymatous. As has been suggested, immediately over the protoxylem points a ray is started. Tangential to this and external to the last formed metaxylem elements, the usual complex of secondary xylem is laid down. Simultaneously, at the point outside the gap between adjacent metaxylem areas is begun another type of ray, as seen in figure 5. There is nothing unusual in subsequent cambial activity, hence it will not be discussed except to describe the resulting tissues.

#### SECONDARY TISSUES

In transverse section the extra-cambial region is marked by conspicuous, elongated, triangularly shaped phloem areas. Alternating with these and opposite the xylem rays are inverted triangularly shaped areas of suberized cells, for the most part isodiametric but occasionally radially elongated. Scattered strands of fibers or single fibers extend more or less longitudinally through these areas. The sieve tubes, with oblique end walls and with elongate-oval sieve faces, are commonly 40-50  $\mu$  in diameter. Their radial walls have well developed lattices with small spots of secondary thickening scattered between the bars of the lattices. In roots up to seven years old there was little evidence of periderm formation; it is recognized, of course, that the canes of this species do not exhibit the same extensive periderm formation and shedding as do other species of *Vitis*. Small oval lenticels occur in longitudinal rows in young roots, but do not seem to attain the proportions and frequency of those of the aerial roots of the unnamed tropical grape (5).

The large vessels form the most conspicuous characteristic of the wood. Many, as in seven year old roots, were 200  $\mu$  or more in diameter. As would be expected these large tracheae are heavily walled, as much as 10  $\mu$  thick. The type of wall thickening is either scalariform or reticulated. Tyloses are fairly common. Fibers, fiber tracheids, and reticulate walled tracheids comprise the bulk of the remaining part of the wood. The meager wood parenchyma is vasicentric. Rays from one to ten cells in thickness and many cells in height are

made up solely of parenchyma. Annual radial growth of wood averaged approximately 1 mm. in the several roots studied.

The walls of the pith cells thicken slightly, but even in the seven year old roots the cells seem to be alive. This is evidenced by the presence of protoplasm and the abundant storage of materials.

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EFFECT OF AFTER-RIPENING TREATMENT ON  
GERMINATION OF WHITE PINE SEEDS  
OF DIFFERENT AGES

HENRY I. BALDWIN

(WITH ONE FIGURE)

Introduction

It has long been a matter of common observation that seeds of white pine (*Pinus strobus* L.) germinate more promptly and completely if first subjected to moist storage at low temperatures, or even if incubated at lower temperatures than are customary in germination tests. Thus JACOBSEN (7) reported better germination outside on a veranda during the winter in Denmark than in the laboratory. BERRY (2) recommended cold storage and PETHERAM (9), fall sowing for white pine. Studies have shown that in a closely related species, western white pine (*P. monticola* D. Don.) (11), and in other western conifers (4, 6) fall sowing is definitely preferable to spring sowing. GRISCH and LAKON (5) have made a comprehensive study of after-ripening of white pine seed, and BARTON (1) reported 69 per cent in 24 days after two months' stratification at 5° C. compared with but 3 per cent in 60 days obtained from untreated controls. Similar results have been reported by the Lake States Forest Experiment Station (12) and by SCHMIDT (10). While fall sowing would seem to be the natural solution of the problem of securing good germination in nursery practice, and is the custom at a number of nurseries, CROCKER (3) recommends stratification for a month or two prior to spring sowing as preferable under some conditions. Since the completion of this paper, KOBLET (8) has reported the results of thorough tests of different temperatures and periods of storage. He found that the suddenness of change from one temperature to another had no influence on the course of germination. Stratification temperatures from 0° to 12° C. were about equally effective, but 9°-12° C. was better than 0°-3° C. The tests reported here differ from the other investigations cited chiefly in dealing with

a number of lots of known age, as well as three samples of unknown age and origin.

### Methods

All germination tests were made on the Jacobsen germinator at a constant temperature of 24° C. Moisture supply, light, and acidity

TABLE I  
AVERAGE GERMINATION OF WHITE PINE SEEDS OF DIFFERENT AGES  
WITH AND WITHOUT STRATIFICATION

LOT NO.	AGE OF SEED AT TIME OF TESTING (YEARS)	GERMINATIVE ENERGY IN 30 DAYS (PERCENTAGE)					PERCENT-AGE INCREASE IN GERMINATIVE ENERGY OF STRATIFIED SEED OVER DRY SEED	STRATIFICATION PERIOD (WEEKS)	
		DRY UN-TREATED SEED	STRATIFIED IN MOIST PEAT AT 8°-10° C.						
			2 WEEKS	4 WEEKS	6 WEEKS	8 WEEKS	16 WEEKS		
1.....	5.5	2.0	6.5	11	.....	.....	450	4	
2.....	4.5	0	0	0	.....	0	0	4	
3.....	4.0	21.5	.....	36	12.5	.....	11	67.5	4
4.....	3.5	9.5	26.5	36	.....	.....	300	4	
5.....	3.0	30.5	.....	.....	67	.....	120	8	
6.....	3.0	21	.....	59	31	.....	59	181	4
7.....	2	30.5	.....	.....	52	.....	70	8	
8.....	2	14.5	.....	74	73	.....	86	410	4
9.....	2	20.5	.....	87	76	.....	94	310	4
10.....	1.5	12.5	51.5	72	.....	.....	500	4	
11.....	1	18.5	.....	.....	92	.....	400	8	
12.....	1	26.5	.....	.....	90	.....	240	8	
13.....	1	16.5	.....	57.5	47.5	.....	72	255	4
14.....	1	36.5	.....	85	85.5	.....	79	133	4
15.....	1	35	.....	97	89.5	.....	90	178	4
16.....	0.5	41	.....	77	.....	.....	88	4	
17.....	0.5	30	.....	13	.....	.....	Decrease	4	
18.....	0.5	19	.....	78	.....	.....	310	4	
19.....	0.2	67.5	.....	93	.....	.....	38	4	
20.....	0.2	59.5	74.5	86	.....	.....	25	4	
21.....	0.2	41.5	.....	68.3	.....	.....	65	6	
22.....	0.2	27.5	69.5	59	.....	.....	119	4	
23.....	?	3.5	.....	18	.....	.....	415	4	
24.....	Probably over 3	.....	.....	.....	.....	.....	.....	.....	
	?	11	.....	67	.....	.....	510	4	
	Probably over 3	.....	.....	.....	.....	.....	.....	.....	

remained constant. No disinfectants were employed. When mold developed the seeds were scrubbed in tap water and wicks and filter papers renewed. The seeds were stratified by mixing thoroughly

with granular peat in small glass jars. These were covered with pieces of cheesecloth held in place with elastic bands. The peat was

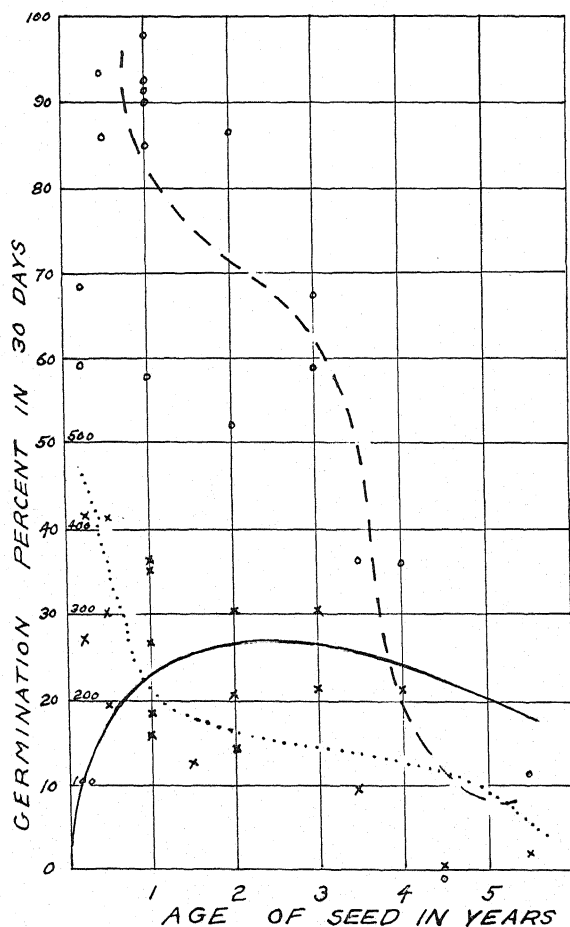


FIG. 1.—Comparison of after-ripened and dry seed, an increase of germination of after-ripened over that of dry seed. Unbroken line, percentage increase; dotted line, dry seed; dashed line, stratified seed.

moistened and stirred over at weekly intervals to insure aeration. The jars were stored in a refrigerator at  $8^{\circ}$ – $10^{\circ}$  C. from two to sixteen weeks, generally four weeks, before being transferred to the germinator. At least two samples of 100 seeds each were used in each test.

### Results and discussion

Table I gives a condensed summary of the results. It is evident that germination was greatly hastened by after-ripening, except in two lots. One of these was probably non-viable seed; at least no germination ever occurred during 60-day tests. In a few cases better germination was secured with longer stratification, but generally four weeks was sufficient to induce very complete germination in a subsequent 30-day period.

A relation between age of seed and effectiveness of after-ripening is not very clear from the data. Apparently the greatest effect was produced in two- to three-year old seed (fig. 1). It might be supposed that the older the seed the more dormant it may have become, especially if stored in a warm place. The data are unsatisfactory in resulting from so many different lots of seed, originating from different seed crops. KOBLET found extreme variability in the behavior of white pine seed of different origin and seed crop, and it is likely that the present data were affected by like influences.

### Summary

Germination tests were made of several lots of white pine (*Pinus strobus* L.) seed of different ages. In most cases after-ripening for several weeks at 8°–10° C. increased the rate of germination markedly. This increase was most prominent in the case of seed two to three years old. Germination decreased with age, that of stratified more rapidly than that of untreated control seed.

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## CURRENT LITERATURE

### Researches on fungi

The fifth volume of BULLER's now classic series<sup>1</sup> is divided into two parts, each distinct in subject matter. Part I, regarded by the author as a continuation of the latter part of the preceding volume, is concerned with the structure and function of the mycelium. Part II is concerned with spore discharge and homologies in *Sphaerobolus* and *Tilletia* (the latter in conjunction with T. C. VANTERPOOL), and with the mechanism of peridiole discharge in *Sphaerobolus*. The numerous illustrations are conveniently located with reference to the text, and are, with one or two exceptions, beautifully clear. A meaty summary, occupying 13 pages, and an exhaustive index, facilitate reference.

Hyphal fusions are shown to be of common occurrence, not only in the assimilative mycelium but also in the fructifications of the Ascomycetes and Basidiomycetes. They are held, in the former case, to facilitate the flow of nutrients, and in the fructification, to unify its structure and increase its mechanical stability. The various types of fusion are analyzed and it is shown that all fusions are between hyphal tips, either of undifferentiated hyphae or of peglike outgrowths. Clamp connections are included as one of the four recognized types of fusion and are regarded as being concerned wholly with growth and nutrition, their homology with the croziers of ascogenous hyphae being denied. In connection with the translocation of protoplasm, emphasis is laid upon the important but neglected work of WAHRlich, and many of his illustrations are reproduced, showing protoplasmic connections between the cells of various fungi. The author describes in great detail the passage of cytoplasm, vacuoles, and nuclei through the pores, and suggests that the phenomena described are adequate to account for the rapid diploidization of haploid mycelium, once its complementary strain is introduced.

It has long been obvious that in the higher fungi large quantities of food must be transported from mycelium to hymenium during the brief period of fructification. BULLER's summary of neglected data and his wealth of additional observations go far to explain how this may be possible. His denial of sexual significance to the clamp connections will carry less conviction, more particularly as some of the facts concerning basidia and clamp connections upon which he bases his judgment, while true for the highly specialized agarics and similar forms, are far less applicable to the Heterobasidiomycetes and to

<sup>1</sup> BULLER, A. H. R., Researches on fungi. Vol. V. pp. xiii+416. figs. 174. Longmans, Green and Co., London. 1933.

the more simplified Homobasidiomycetes which must be regarded as transitional. Nor is adequate weight given, in the opinion of the reviewer, to the effect of the overwhelmingly increased importance of the diploid generation in Basidiomycetes as compared with Ascomycetes.

The studies on *Sporobolomyces* confirm the facts previously reported concerning this interesting yeastlike genus, and add to them certain particulars, notably the capacity of a single cell to produce up to three spores in succession on the same sterigma, or to produce three or four spores on separate or branched sterigmata. The author argues convincingly for the inclusion of the genus among the Basidiomycetes. Passing mention is made of BREFELD'S observation of similar behavior in the basidiospores of certain of the tremellaceous fungi, but this is not stressed, and recent studies showing that this phenomenon is widespread and common in that group are not mentioned. The habit of germination by repetition in moist air and of budding in yeastlike fashion in nutrient solutions, so characteristic of the basidiospores of the Tremellales, would seem to justify the conclusion not only that *Sporobolomyces* and *Bullera* are Basidiomycetes, but that they are imperfect members of the Tremellales.

A summary of the studies on *Tilletia* was published some years ago and is familiar to mycologists. As a result of their studies, it will be recalled, BULLER and VANTERPOOL concluded that the so-called spores of *Tilletia* are in reality greatly modified sterigmata, and the so-called conidia, primary and secondary basidiospores. The evidence for this point of view is given in great detail. But here again, adequate recognition of the functions and variations of the epibasidium and of the phenomenon of germination by repetition in the Heterobasidiomycetes might make the homology even more convincing.

The last chapter is devoted to a study of *Sphaerobolus*, including its range, kinetics, and ecological relationships. The genus is regarded as primarily coprophilous and only secondarily xylophilous. In view of the great abundance of *Sphaerobolus* on wood in localities to which herbivorous animals have no access, this point of view seems overstressed.

As is the case with previous volumes, the wealth of information it contains and the painstaking and exact attention to details make this a reference work which all mycologists must not merely read, but must keep within reach for constant reference. Its very importance, however, imposes critical appraisal of the conclusions which it presents.—G. W. MARTIN.

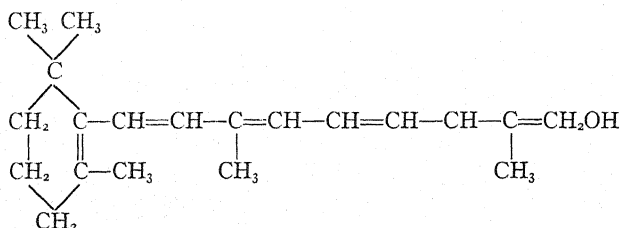
#### Carotenoids

Until a few years ago the chemical constitution of the carotenoids was essentially unknown. While empirical formulae for carotene and xanthophyll had been determined as  $C_{40}H_{56}$  and  $C_{40}H_{56}O_2$  respectively, these formulae gave no indication of the structural pattern of the molecules. During the last few years great progress has been made, and we can now consider the structural formulae of the carotenoids as fairly well established. In addition, the long suspected relationship between the yellow pigments and vitamin A has been



The arrow indicates the center of the molecule, the two halves of the molecule being arranged symmetrically on opposite sides of the double bond.

Vitamin A is produced by the symmetrical breaking of the molecule at this center double bond, with the absorption of water to form a primary alcohol. The molecule of vitamin A is



The final section of the monograph contains 50 beautiful photomicrographs of the various crystallized carotenoids. A bibliography of 28 pages emphasizes the recent growth of carotenoid literature, for most of the papers have come out within the last five years.—C. A. SHULL.

#### Electrokinetic phenomena

Since the discovery of electroosmosis by REUSS in 1808, many observations have been made on biological materials which involve the electrical forces which reside on the surfaces of these colloidal substances. An important monograph on electrokinetic phenomena and their application to biological phenomena has been prepared by ABRAMSON<sup>3</sup> and published as monograph no. 66 of the American Chemical Society Monograph series.

The first chapter is a valuable historical summary of the discoveries during the period from the time of REUSS's work in 1808 to the formulation of the theory of electrical double layers by HELMHOLTZ in 1879. This is followed by a presentation of the HELMHOLTZ theory and its application to various phenomena such as electrophoresis, streaming potentials, viscous flow, sedimentation potentials, etc., and the early experiments which seemed to offer sufficient confirmation of the HELMHOLTZ hypothesis.

The third chapter considers the methods now in use in such experimental work. The use of ultramicroscopes, the problems of illumination, magnification, rotating and alternating fields, gas bubbles and liquid droplets, moving boundaries, streaming potentials in capillaries and through diaphragms, and methods of studying electroosmosis and sedimentation are discussed in illuminating manner. The fourth chapter considers the more recent theories such as those

<sup>3</sup> ABRAMSON, H. A., *Electrokinetic phenomena and their application to biology and medicine*. 8vo. pp. 331. figs. 106. Chemical Catalog Co., New York. 1934. \$7.50.

of GOUY, and of DEBYE and HÜCKEL. The succeeding chapters concern specific biological systems and general surface phenomena. The proteins and related compounds such as hemoglobin, pepsin, etc., are taken up first, followed by consideration of salt effects on inert surfaces and inorganic surfaces in general. Glass, quartz, silica gel, clays, metallic colloids, metallic oxides, sulphides, carbon particles, etc. claim attention in this part of the work. Then the organic surfaces are considered, such as organic emulsoids, paraffin oils, phenol, guaiacol, benzonitrile, and aniline in aqueous media, lipide emulsions of many kinds, cellulose, amylose, glycogen, etc. Gases in colloidal form are discussed in a brief chapter; and finally such biological systems as blood cells, spermatozoa, frog skin, human skin, tooth enamel, trout eggs, onion skin, bacterial cells, anti-bodies, viruses, and related systems receive careful treatment.

Appendix I gives a list of symbols used in the mathematical discussion of potentials, and II contains a list of constants and conversion factors which are necessary in applying the formulae to specific problems. Appendix III refers to patents which have been granted on processes involving electrokinetics, such as drying of peat, electrodeposition of latex, purification of clay, water, sugar solutions, serum, and gases, separation of water and oil emulsions, and the impregnation and tanning of hides in leather manufacture.

The presentation is technical enough so that the reader must bring some knowledge of the field to the task of reading it. Nevertheless the reviewer feels that ABRAMSON has performed a real service to biologists in bringing these important and difficult problems into the compass of a relatively brief monograph. In masterly fashion he has drawn into a well connected presentation material from widely separated fields of physics, chemistry, and biology. Much of the material would remain obscure and inaccessible to biologists if not brought together by someone who understands the relationships involved. Electrokinetic phenomena are of great significance in biology, and this book will help to focus attention upon problems which have been overlooked and neglected in the past. Many investigators in the biological field will find the information in this monograph extremely useful.—C. A. SHULL.

#### Families of monocotyledons

In the review<sup>4</sup> of the first volume of this work,<sup>5</sup> it was predicted that it would be more widely noted and would stimulate more interest in the possible relationships among families of angiosperms than have many of the systems of classification which have appeared from time to time. The fulfilment of this

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<sup>4</sup> BOT. GAZ. 82: III-III. 1926.

<sup>5</sup> HUTCHINSON, J., The families of flowering plants. Vol. II. Monocotyledons. Arranged according to a new system based on their probable phylogeny. 8vo. pp. xiv+243. figs. 107. Macmillan & Co., London. 1934. \$6.

prophecy in the United States is shown in the use of HUTCHINSON's classification of the dicotyledons in taxonomy courses in several colleges. Many botanists have been awaiting with keen anticipation the appearance of this companion volume on the monocotyledons.

The author's preface states that "the principal object of the book is to provide the student with descriptions of the families of monocotyledons arranged in as logical a sequence as may be possible according to their probable phylogeny," and that "although somewhat drastic alterations are proposed, it should be understood that the work is not monographic, but represents only the beginning of an endeavour to establish a phylogenetic system for the monocotyledons." Nevertheless, HUTCHINSON has been able, during the eight years in which much of his attention has been concentrated on the study of the living and dried specimens at Kew, to build up a truly thought-provoking system. His treatment departs from that of BENTHAM and HOOKER even more than was true of his system of classification for the dicotyledons.

The group is considered to be monophyletic, showing close relationship to the dicotyledons only through the Butomales and Alismatales. These share with the Ranales an apocarpous gynoecium and a tendency to possess numerous stamens. Endosperm, which is regarded as a primitive feature in general, has been lost in these lower forms, probably owing to the adoption of an aquatic habit. These primitive stocks are placed with, and are considered to have given rise to, all the forms included under the first general division of the Calyciferae (calyx-bearers). These comprise, among others, the Commelinales, Bromeliales, Zingiberales, Xyridales, Eriocaulales, Potamogetonales, and Najadales. These are mostly forms of moist or aquatic habitat which either retain a biseriate perianth or show marked perianth reduction and sexual differentiation.

Somewhere from these primitive stocks, possibly from forms resembling *Scheuchzeria*, there arose a more terrestrial race, including such forms as the more primitive of the existing Liliaceae. This underwent prolific evolution to give rise to the remainder of the monocotyledons. Many of these, including the Liliales, Palmales, Burmanniales, Orchidales, Arales, Typhales, etc., are grouped by HUTCHINSON into a second great division, the Corolliferae (corolla-bearers).

A third climax group, the Glumiflorae, is thought to have branched off independently from the Liliaceous stock. It includes the Juncales, which in turn have given rise to the Cyperales and Graminales.

Conservative botanists will be startled at HUTCHINSON's treatment of the Liliaceous complex, especially the old groups of Liliaceae and Amaryllidaceae. Not only is his tendency to the reduction in size of families carried into effect with the recognition of the Trilliaceae, Smilacaceae, Ruscaceae, Agavaceae, Hypoxidaceae, and others, but he regards the type of inflorescence as of greater importance than epigyny or hypogyny. This results in a transfer of the tribes Agapantheae, Allieae, and Gilliesieae from the Liliaceae to the Amaryllidaceae. The latter thus becomes, according to HUTCHINSON, "a very homogeneous and

natural group, the most distinctive and constant feature of which is the umbellate, scapose inflorescence." The family as thus constituted is given ordinal rank as well, as are several other single families.

The orders and families recognized and described in this volume total 29 and 68 respectively, bringing HUTCHINSON's total for the angiosperms up to 105 and 332.

The usefulness of the book is much increased by the inclusion of keys to the genera of all families except the Orchidaceae and Gramineae, in addition to a well planned artificial key to the families. Other desirable features, which were found in the preceding volume as well, are the numerous drawings, many of them original, of species which exhibit points of special phylogenetic interest. Sketch maps, showing the distribution of certain families, are also included.

HUTCHINSON acknowledges the assistance of Messrs. DANDY, HUBBARD, and SUMMERHAYES in the treatment of the Hydrocharitaceae, Gramineae, and Orchidaceae respectively. He most appropriately dedicates this volume to Dr. AGNES ARBER.—C. E. OLMSTED.

#### Gramineae

In the publication of this book<sup>6</sup> ARBER has brought together in one volume the results of many years of research devoted to the study of the monocotyledons, and of the grasses in particular. Admittedly no attempt has been made to include within the pages of one book all the facts and theories regarding such a large plant group. Instead, the author has limited herself to "those aspects of the subject which happen to make the greatest appeal. . . ." Thus emphasis naturally falls upon the morphological treatment of the grasses, to which subject the author has devoted the major portion of her research. Humanistic, historical, and phylogenetic relationships receive a rather extended treatment while references to ecological, physiological, and taxonomic problems are more or less incidental.

The initial chapters are devoted to the humanistic, historical, and geographical phases of the grasses. In these, man's discovery and use of the grasses and their relation to the history of mankind are recounted. The cereal grasses, those used for pasture, sugar, and scent are considered in order.

Following this section are four chapters which treat the bamboos with respect to their vegetative phase, tree habit, reproductive phase, and spikelet and fruit. In discussing the tree habit, ARBER takes exception to the theory of the arboreal ancestry of the angiosperms, and suggests that this habit may be regarded as "an expression of racial senile degeneration . . . as the final expression of a certain fundamental tendency in plant life." This she explains may be due to the development of the cellulose wall and the consequent loss of plasticity; and she concludes her argument with the question, "May we not then visualize the

<sup>6</sup> ARBER, AGNES, *The Gramineae: A study of cereal, bamboo, and grass*. pp. xvii+480. figs. 212. University Press, Cambridge and Macmillan Co., New York. 1934. \$8.50.



tree habit as the ultimate expression of the liability to the accumulation of inert organic matter—a tendency which can be kept within bounds in the youthful phases of a race, but which is apt to pass out of control when senescence is reached?" In connection with the reproductive phases of bamboo, there is an interesting discussion of the phenomenon of gregarious blooming and the causes of periodic flowering.

The life cycle, reproductive and vegetative phases, embryology and morphology of the grasses are the subjects to which the major portion of the volume is devoted. The results of the author's extensive research are brought together and to some degree correlated with the work of other investigators. These chapters, as well as all others, are liberally foot-noted and there is an accompanying bibliography which includes most of the important contributions on the Gramineae.

The interpretation of such structures as the coleoptile, mesocotyl, and epiblast will doubtless provoke some controversy, for it is to be expected that workers in such a large plant group as this will be at variance in respect to many of the morphological conclusions presented. But there is a refreshing and stimulating unorthodoxy in the treatment of some of our formal morphological concepts, such as the impossibility of a leaf being terminal to a stem, the use of the term adventitious, and the question of root and shoot terminology. In this latter connection, the author concludes that "stem and leaf cannot be accepted as valid morphological categories."

The final chapters deal with problems of the distribution and dispersal, hybridization, and the pattern and rhythm of the grasses.

The declared purpose of this volume was "to detect the pattern and rhythm underlying that complex of plant types called the Gramineae." To the solution of this problem ARBER has contributed valuable data and critical thought; yet, in a sense, she confesses partial failure, for this excellent treatise concludes with the question and statement: "What is the *meaning* of the differences that separate the Gramineae so delicately, yet so definitely, from any other order, and that so prevail that a grass remains a grass, however freely that type may vary? . . . The mystery abides."

But though the controversial aspects of grass anatomy and phylogeny may remain unsettled, there is no question but that this volume is an important and distinguished contribution to botanical literature, a treatise that will be generally recognized as a standard in its field. It is well illustrated, clearly written, and will undoubtedly be received with appreciation by all botanists interested in this phase of plant science.—H. E. HAYWARD.

#### Identification of commercial timbers

A new volume in the American Forestry series<sup>7</sup> devoted to the identification of commercial timbers of the United States has recently been published. This

<sup>7</sup> BROWN, H. P., and PANSHIN, A. J., Identification of the commercial timbers of the United States. pp. xxvi+223. Illustrated. McGraw-Hill Book Co., New York. 1934.

will be a welcome addition to the literature relating to those phases of forestry that deal with the anatomy and identification of our native commercial woods. The book is specifically designed to meet the practical needs of the college student, the forester, and the workers in industries dealing in wood products.

The organization of the book is logical, and consists of three major divisions. The first deals with the anatomy of woods and includes a discussion of the general concepts of tree growth, the physical properties of wood, the gross anatomical features of wood, and a concluding section on minute anatomy. While this section of the text is somewhat more brief than might be desired, it should afford sufficient information to enable the worker to use the keys for identification successfully. It is supplemented by a comprehensive glossary which includes all the terms referred to in the keys and descriptive portions of the text.

The second section of the volume consists of two keys for the identification of the commercial timbers of the United States. The first key is based on characters that can be discerned with the naked eye or with the aid of a hand lens (10 $\times$ ). The second key is based upon minute characters which require the use of a compound microscope. Accompanying the first key are photomicrographs of the cross sections of the woods (5 $\times$ ) while the key based on the microscopic characters is illustrated with cross and tangential sections (75 $\times$ ). The nomenclature used in the keys includes the common trade names as well as the scientific names.

The final section is devoted to descriptions of the species referred to in the keys. Each species is described in terms of its general characteristics and its minute anatomy. For the convenience of the reader, the plate numbers of each species are noted in connection with the description.

The uniformity of the plates and the general excellence of their reproduction together with the inclusion of tangential sections in each case add greatly to the value of the work.—H. E. HAYWARD.

#### Economic plants

A volume<sup>8</sup> has just appeared which provides the teacher of botany with a long needed work on the economic uses of plants. The increasing tendency to correlate the teaching of pure plant science with the applied and with the activities of everyday life has revealed a dearth of adequate material suitable for textual and collateral reading. The present volume is well designed to meet this need, and should also prove valuable as a source book for teachers in the high schools and the upper grades.

The volume is well indexed and the organization is logical. The introductory chapters deal with "the plant kingdom" and "cells, tissues, and organs." These are designed to orient the reader with respect to the major groups in

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<sup>8</sup> STANFORD, E. E., *Economic plants*. pp. xxiii+571. figs. 376. Appleton-Century Co., New York. 1934. \$4.50.

plant classification, plant nomenclature, and to provide the fundamental concepts and terminology of plant anatomy as well as the functions of the major plant organs.

The discussion of the economics of plants is divided into five major sections: (1) forest products, under which are chapters dealing with the composition and structure of wood; uses of wood; resins, tanning, and cork; rubber and latex products; (2) textile plants; (3) paper and pulp; (4) food products including cereal crops, sugar plants, fixed oils (not all food products), nitrogen products, fruits, spices and beverage plants; (5) medicinal plants.

The treatment of the subject matter is in part historical and geographical as well as economic. It is written in an interesting and readable style and is generously illustrated.

The scope of the book is so great that the discussion of many of the topics is necessarily brief. For this reason it is to be regretted that the author did not include lists for supplementary reading, either in connection with each chapter or as an appendix to the volume as a whole. This would have given added value to a work which in other respects is a worthwhile contribution to current botanical literature.—H. E. HAYWARD.

#### Wood identification

The value of RECORD's books on wood structure has been proved by the exhaustion of two editions of his earlier book, *Economic woods of the United States*, a notice of which appeared in this journal.<sup>9</sup>

A larger volume<sup>10</sup> has now appeared which is much more than a third edition. The first half of the book is entirely rewritten and much material has been added to the second part. It seems safe to say that the many who have found the earlier editions so useful will be even better pleased with the present book.

There has always been more or less confusion in the use of terms used to describe various details of wood structure. It is therefore gratifying to find the book using the terms recommended by the International Association of Wood Anatomists.<sup>11</sup> These terms have been defined in somewhat more detail than in the original report, and have received a practical application in the descriptions. The number of illustrations has been increased and many good photomicrographs have been included. One of the notable features of the book is an artificial key for the identification of 80 economic woods of the United States and Canada. Structures visible to the naked eye and those revealed by the use of a hand lens and by a compound microscope are the criteria for the identifica-

<sup>9</sup> BOT. GAZ. 68:480. 1919.

<sup>10</sup> RECORD, S. J., *Identification of the timbers of temperate North America*. 8vo. pp. 196. pls. 6; figs. 47. John Wiley & Sons, New York. 1934. \$3.

<sup>11</sup> COMMITTEE ON NOMENCLATURE: International Assn. of Wood Anatomists. *Glossary of terms used in describing woods*. Tropical Woods no. 36. 1-12. 1933.

tions. Experience has shown this key to be reliable but to require a very considerable background of experience on the part of the operator.—G. D. FULLER.

#### Raunkiaer's life forms

Perhaps the most important book of the year for plant ecologists and phytogeographers is the recent volume containing the collected papers of RAUNKIAER, translated into the English language.<sup>12</sup> The first of this series of contributions appeared over 30 years ago (1904), and like most of this author's papers, was in the Danish language. It was a preliminary statement of a classification of plants based on their adaptations to survive the unfavorable season. This arrangement gave a series of groups that soon became known as "Raunkiaer's life forms." This concept, together with its application in the form of "biological spectra," used to characterize the phytogeographical climates of the earth, has been RAUNKIAER's most notable contribution to plant ecology. It was promptly accepted by European ecologists and has become familiar to American investigators through translations into English of portions of RAUNKIAER's writings and through a later paper in German by RAUNKIAER himself.

Scarcely less notable than RAUNKIAER's life forms has been his contribution to the statistical study of plant communities. His methods of determining the degrees of frequency (valency) of the various members of the communities, now so widely used, were described in detail in a Danish paper which appeared in 1909 and in a French article dated 1918. Probably RAUNKIAER's contributions have done more to promote quantitative investigations in plant ecology than have those of any other ecologist. In this respect he has proved a worthy follower of WARMING.

The third notable contribution of this versatile scientist is his studies of the vegetation of the Mediterranean region which occupy not less than 100 pages of the present volume, including an article of 75 pages hitherto unpublished.

The translation of the Danish articles by H. GILBERT-CARTER of Cambridge, and Miss A. FANSBØLL has been well done, as has that of the German and French papers by A. G. TANSLEY, who has edited the whole volume. The Danish scientists in charge of the work were K. GRAM, H. MOLHOLM HANSEN, OVE PAULSEN, JOH. GRØNTVED, and the late C. H. OSTENFELD. To all of these English speaking ecologists we are indebted for a monumental volume which is certain to make the notable contributions of RAUNKIAER more widely and fully appreciated.—G. D. FULLER.

#### Flora of Buffalo and vicinity

Residents of Buffalo and vicinity and students of plant distribution will be interested in the present volume,<sup>13</sup> which is a floristic study of the vascular

<sup>12</sup> RAUNKIAER, C., *The life forms of plants and statistical plant geography, being the collected papers of C. RAUNKIAER.* pp. xvi+632. figs. 189; portrait. Clarendon Press, Oxford. 1934. \$14.

<sup>13</sup> ZENKERT, C. A., *The flora of the Niagara frontier region. Ferns and flowering plants of Buffalo, N. Y., and vicinity.* Bull. Buffalo Soc. Nat. Sci. vol. XVI. pp. x+328. Illustrated. Buffalo Museum of Science, Buffalo, New York. 1934. \$2.

plants found in the area within a radius of about 50 miles of Buffalo. The body of the work falls into four main sections: an historical account of plant exploration in the area; a discussion of the regional environmental conditions; an annotated list of the 1187 native and 400 introduced species of the region; and a description of the principal ecological areas and plant societies. The work is copiously illustrated.—C. E. OLMSTED.

#### Tropical and Japanese plant observations

In a recently published memoir<sup>14</sup> a great many references are made to a visit to Buitenzorg in 1898 and another visit to India in 1928–29, which the author of the memoir spent at the Institute for Plant Physiology of Sir JAGADI CHANDRA BOSE. The main researches of his first trip were on the formation of indigo, of palm wine, and on the secretion of water by liana stems. These studies were continued and enlarged during MOLISCH's second trip to the tropics, and can be found discussed in his book, *A Naturalist in India* (1930).

Many other observations along the line of plant physiology were made during his prolonged stay in Japan, from 1922 to 1925, and are gathered in his book, *Plant Physiology in Japan on the Basis of Personal Observations* (1926).

MOLISCH writes in German but his diction is clear and concise and can easily be read by English speaking students who have some knowledge of German.—A. C. NOÉ.

#### Haemolytic systems

Monograph no. 6 of the Protoplasma Monographien series is a volume by PONDER<sup>15</sup> on red blood cells and haemolytic phenomena. There are ten chapters, dealing with counting methods, dimensions, shape and structure, and chemical composition and metabolism; permeability and osmotic haemolysis; properties of simple haemolytic systems; inhibition and acceleration of haemolysis; resistance series; systems containing sensitizing agents; and miscellaneous forms of haemolysis. There are several appendices, on sedimentation, rouleaux formation, stromatolysis, and the erythrocytes of the Camelidae. An extensive bibliography and subject and author indices are included.—C. A. SHULL.

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Copies of the photograph of Professor H. C. COWLES, which appeared as a frontispiece in the September issue, may be secured by addressing the Editorial Office of the *Botanical Gazette*, University of Chicago, and inclosing stamps to cover cost and mailing. One copy, 18¢; each additional copy, 10¢.

<sup>14</sup> MOLISCH, HANS, *Erinnerungen und Welteindrücke eines Naturforschers*. pp. 232. Emil Haim, Wien and Leipzig. 1934. RM 9.

<sup>15</sup> PONDER, ERIC, *The mammalian red cell and the properties of haemolytic systems*. 8vo. pp. xii+311. figs. 52. Gebrüder Borntraeger, Berlin, 1934. RM 22.5.

# THE BOTANICAL GAZETTE

*March 1935*

## RELATION OF ROOT DISTRIBUTION TO ORGANIC MATTER IN PRAIRIE SOIL<sup>1</sup>

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(WITH ELEVEN FIGURES)

### Introduction

Although the root distribution of the chief grasses and forbs of tall-grass prairie has been known for 15 years (18), no studies on the relation of root distribution to the organic matter in the soil have been made. In fact, so far as the writers are aware, few studies of this type have been undertaken in America. SPRAGUE (15), working in New Jersey, has attempted to correlate root occupation of the several soil layers with their specific soil properties. The grasses, chiefly Kentucky bluegrass and Colonial bent grass, were grown on a formerly cultivated soil of the gray-brown forest soil group of the humid eastern states. Practically all of the roots were found in the upper 9 inches of soil, the abundance decreasing rapidly with depth. He found no correlation between root distribution and organic carbon content of the soil.

During the late fall of 1932, the writers selected a representative square meter of typical grassland in each of the two most important types or consociations of tall-grass prairie. The soil was removed to the depth of penetration of the grasses for the purpose of determining the relation of root distribution to the organic matter in prairie soil.

<sup>1</sup> Contribution from the Department of Botany, University of Nebraska, no. 87.

<sup>2</sup> Department of Botany.

<sup>3</sup> Department of Agronomy.

The roots were separated from the soil and the organic matter in both roots and soil in the several soil levels was determined.

### Stations

Little bluestem (*Andropogon scoparius*) and big bluestem (*A. furcatus*) are the most important two of the six grassland types occurring in the eastern one-third of Nebraska and the western one-third of Iowa, but also including large areas in Kansas, Missouri, Minnesota, and South Dakota (19). These two consociations constitute fully 80 per cent of the tall-grass prairie of this region. Because of their importance, the stations were selected in these types.

A tract of 120 acres of upland prairie dominated by little bluestem and known as the Belmont prairie lies one mile north of Lincoln. The vegetation has been thoroughly studied and continuous records of the environmental factors during the growing season have been obtained over a period of 15 years (20).

An area of about 40 acres of nearly pure big bluestem prairie is located on the floodplain of the Missouri River near Union, about 40 miles east of Lincoln, Nebraska. This prairie, owned by Mr. George Everett, was selected as representative of the *Andropogon furcatus* consociation and carefully studied (7, 19).

### Vegetation

The Belmont prairie covers rolling hills. Little bluestem constitutes about 60 per cent of the vegetation; needle grass (*Stipa spartea*), June grass (*Koeleria cristata*), and prairie drop seed (*Sporobolus heterolepis*) are other common bunch grasses. Big bluestem and Indian grass (*Sorghastrum nutans*) are important sod-forming species as is also Kentucky bluegrass (*Poa pratensis*) (fig. 1). Scribner's panic grass (*Panicum scribnerianum*) and Wilcox's panic grass (*P. wilcoxianum*) are common interstitial species. The basal or ground cover is about 15 per cent, although the foliage cover, which varies greatly from year to year and with the progress of the season, is usually between 75 and 100 per cent (fig. 2). Although the abundant forbs often form extensive societies, the grasses are everywhere the dominant species. In some local areas forbs are sparingly represented.



FIGS. 1, 2.—Fig. 1 (above), view in the *Andropogon scoparius* type of grassland late in June. Grass is about 15 inches high and *Psoralea floribunda* about 30 inches. Fig. 2 (below), detail of basal cover in upland prairie. Bunches of little bluestem have been cut and reveal about 85 per cent soil surface.



The Everett prairie occurs on nearly level, well drained land lying between the Missouri River and its western bluff (fig. 3). Big bluestem alone constitutes 85 to 90 per cent of the vegetation. The chief accompanying grasses are Indian grass (*Sorghastrum nutans*), tall panic grass (*Panicum virgatum*), and relict slough grass (*Spartina michauxiana*). As a result of continued annual mowing, bluegrass has invaded but only in relatively small amounts. These sod-forming grasses, with a sprinkling of forbs, form a tall, dense foliage cover although the basal cover is only about 12 per cent (fig. 4).

### Growth

Growth of vegetation on both upland and lowland prairie is normally renewed about the middle of April, although certain species are somewhat earlier. Perhaps only 2 per cent of the vegetation consists of annual plants. The abundant accumulations of reserve foods in the underground parts of the perennials permit rapid development with the occurrence of warm weather in spring. Little bluestem attains a height of 3 to 4 inches early in May and a general level of about 18 inches late in July. The flower stalks then elongate rapidly and reach a height of 2 to 2.5 feet. FLORY (6) has determined its average percentage of growth by weight during a period of 3 years as follows: 2 per cent in April, 28 in May, 36 in June, and 21 in July. By the end of July it had practically completed vegetative growth and had the greatest functional surface of leaves. The 12 per cent of growth in August and the 1 per cent in September consisted almost entirely of flower-stalk production.

The roots of the little bluestem reach a depth of about 4 feet. The extent of deterioration of the old root system each year and the rate of its replacement as well as the addition of more roots are as yet unsolved problems. It is known, however, that an entirely new root system may develop to the usual depth in a single summer if a block of sod is transplanted (4). Upland grasses and forbs in general grow more slowly than do those of lowlands and they are of smaller stature.

Growth in the low prairie is rapid. Big bluestem, the most important dominant, elongates at a maximum rate of nearly 2 cm. per day, and like the other tall grasses completes its vegetative growth



FIGS. 3, 4.—Fig. 3 (above), general view of Everett prairie late in June. Grass is *Andropogon furcatus* and coarse herb, *Silphium integrifolium*. Fig. 4 (below), basal cover of a practically pure stand of *A. furcatus* on low prairie near Union, Nebraska. Plants cut and photographed late in June.

late in July. Then the flower stalks begin to develop, often elongating 4 to 8 cm. per day, and the plant reaches a height of 6 to 10 feet. The roots extend to near the water table at 7 to 8 feet. Other tall grasses grow at a similar rate, slough grass always exceeding the bluestem in height. The taller forbs grow even more rapidly than the grasses and continuously overtop them. The yield of hay ranges from 0.75 to 1 ton per acre in the little bluestem type; that of the big bluestem prairie is 1.5 tons or more per acre. The vegetation cut from the square meter of upland soil before root excavation had a dry weight of 438 gm.; that from the lowland 1135 gm. Grasses constituted 92 per cent of the yield from the upland quadrat and 94 per cent of that from the low prairie.

#### Habitat factors

The mean annual precipitation at Lincoln is about 28 inches; that at Nebraska City, a few miles south of the Everett prairie, is 34 inches. Its distribution is of the Great Plains type, between 76 and 79 per cent occurring between April 1 and September 30.

Water content in the surface 6 inches of upland soil varies widely and rapidly, often 10 per cent or more during a single week. It was reduced to less than 5 per cent in excess of the hygroscopic coefficient one to four times annually during eleven of the twelve years that samples were regularly taken (20). Only twice during this period was the water content of this layer reduced to the hygroscopic coefficient. At greater depths to 4 feet there was always some water in excess of the hygroscopic coefficient, the available supply usually ranging between 5 and 20 per cent. The greater amounts of available soil moisture at the Everett prairie during a year of approximately average rainfall are shown in figure 5.

The average day humidity at Belmont, during a period of 12 years, varied between 50 and 80 per cent during years of greater rainfall but fell frequently to 40 to 50 per cent during drier years. In the Everett prairie it was 10 to 15 per cent higher.

Evaporation from white, cylindrical, porous cup atmometers varied greatly from year to year. It rarely fell below an average weekly loss of 10 cc. per day and was usually between 20 and 30 cc.; during periods of drought it sometimes reached 40 to 55 cc. The

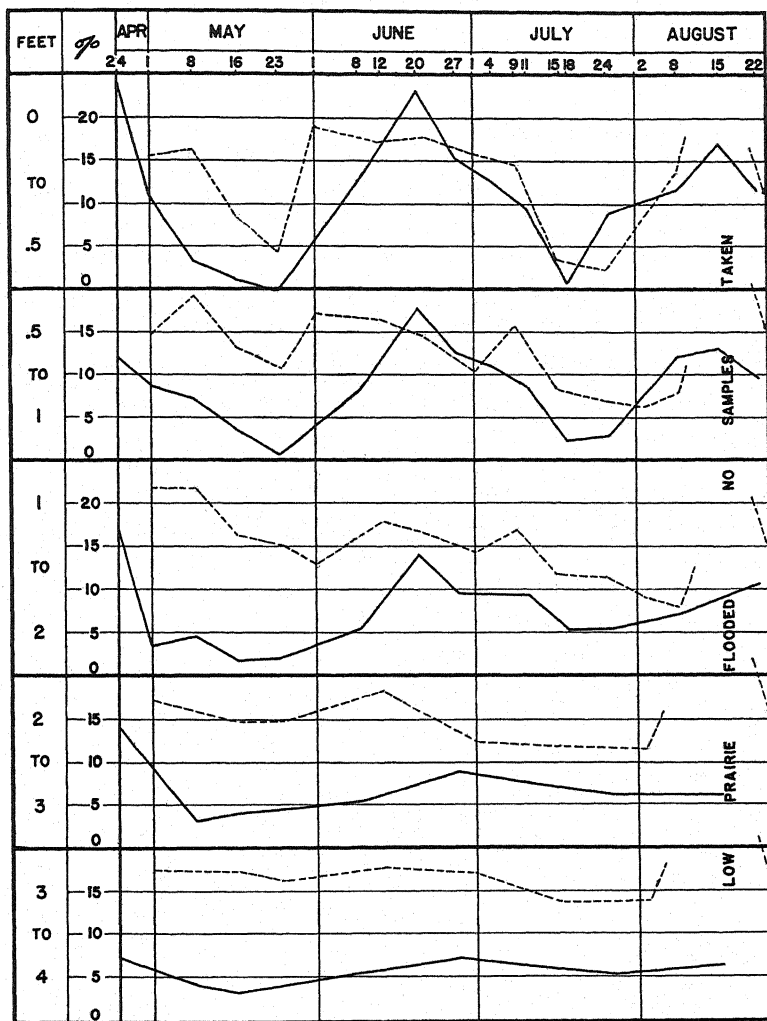


FIG. 5.—Percentage of water in excess of hygroscopic coefficient at Belmont station (solid line) and Everett prairie (broken line) at several depths during 1932.

average daily evaporation rate was considerably less in the lowland prairie at Union, as was also the wind movement.

### Soils

The soil at the Belmont station is Lancaster loam, a mature upland soil of rolling topography derived from the Dakota sandstone formation. The samples were taken from a gentle north slope. The *A* horizon extends to a depth of about 16 inches; the *B* horizon lies between 18 and 30 inches; and the *C* horizon extends far beyond the range of penetration of the roots of the grasses. The surface soil is a very dark grayish brown loam about 12 inches in depth, thoroughly interlaced with grass roots, well supplied with organic matter, and has a highly developed fine granular structure. It contains noticeable amounts of medium and fine sand and is very friable. The soil at 12 to 16 inches forms a transition layer, grayish brown in color, friable, increasing in compactness, clay content, and size of structural aggregates, the latter grading up to 4 or 5 mm. in size. Below this is the horizon of clay accumulation which extends to a depth of 30 inches. It is friable, light grayish brown in color, and shows occasional rusty brown iron stains. Grass roots are numerous but small. The texture becomes much more sandy from the 30-inch depth to about 42 inches. There is little change in color except that rust stains and chalky spots are numerous. At the 42- to 44-inch level the sand is entirely rust-brown in color. Below this it is light grayish brown and is compact and cemented.

The soil of the Everett prairie is mapped as Wabash silt loam, a type frequently found in the bottomlands of eastern Nebraska. It is described as a dark grayish brown or dark brown to black, heavy, smooth silt loam, having an average depth of 24 inches, underlain by a more compact silt loam which is usually somewhat lighter in color (8). It is alluvial in origin; the topography is flat and the drainage was originally poor, but has been greatly improved by cleaning the drainage channels and constructing roads and ditches. Before the land was settled it was undoubtedly subject to frequent inundations by surface run-off from the higher land, and has been inundated, once to a depth of several feet, within the past 50 years. Mr. Everett has owned this prairie for more than 50 years and has seen

the vegetation gradually change, as the result of improved drainage conditions, from slough grass to big bluestem.

The profile examined was found to have a surface horizon, about 6 inches in depth, of dark grayish brown clay loam having a fine granular structure owing to the action of the fibrous grass roots with which this layer is heavily interspersed. Although this soil is mapped as silt loam, the high hygroscopic coefficients (18.2 and 19.7 for the first and second 6 inches respectively) indicate it to be much heavier in texture. It is a heavy clay loam. The 6- to 12-inch layer is less granular, lighter in color, and much lower in organic matter than the surface 6 inches. At the depth of 12 inches there is a layer one-eighth to one-fourth inch thick of very light grayish brown material, sandier than the deposits above and below.

At the depth of 12 to 24 inches there is a horizon whose nearly black color, highly developed fine granular structure, and comparatively great thickness indicate that it was formerly the surface soil for a long period of time. It has the morphological characteristics of a surface soil more fully developed than has the present surface foot. The physical and chemical analyses confirmed this field observation. Compared with the 6- to 12-inch horizon, the second foot was found to have smaller volume-weight and specific gravity and greater pore space, organic matter, and nitrogen content. The hygroscopic coefficient, 15.6, indicates it to be somewhat less heavy in texture than the surface layers. It is believed that many decades have elapsed since it was first covered with the present surface soil.

Between 24 and 28 inches there is a transition to a zone of light grayish brown soil which extends from 28 to 45 inches. A few gray and rust-brown mottlings are present, indicating imperfect drainage. Grass roots are in evidence, but structural units are only slightly developed. At 45 inches there is a rather abrupt transition to a dark gray material of massive structure containing somewhat more very fine sand and silt than the horizons above it. Within this horizon, which extends from 45 inches downward to the ground-water level, are strata of slightly sandier texture and lighter gray color. The darker color and slightly higher organic content indicate that the 45-inch horizon probably was once a surface soil for a long enough time to accumulate such a supply of organic matter. The slate-gray

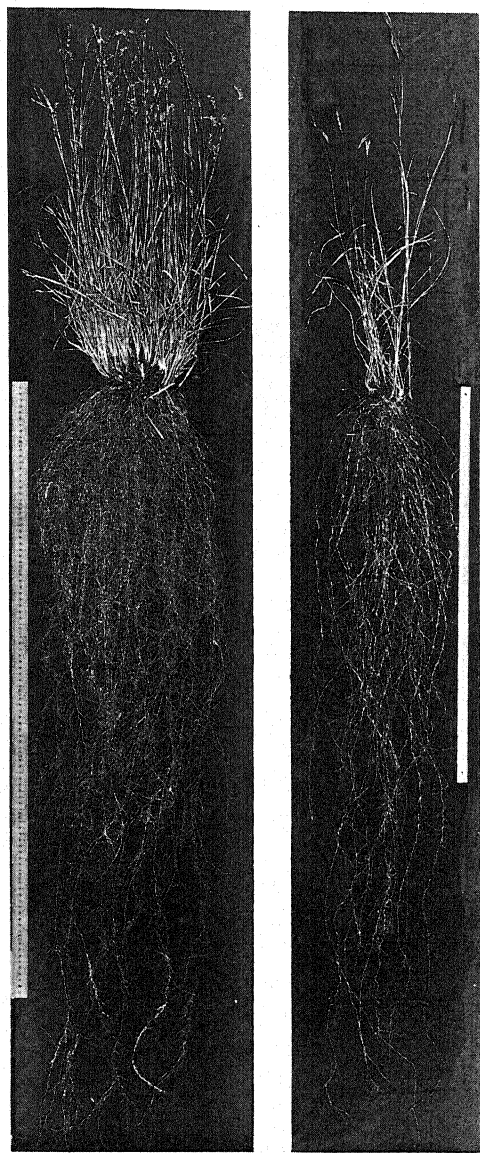
color indicates the deficiency of aeration and drainage, but the soil is now well penetrated by the roots of both grasses and non-grasses and the channels left by the decay of former generations of roots.

### Root habits of plants excavated

The vegetation on the square meter selected for the excavation in the little bluestem prairie was about 87 per cent *Andropogon scoparius*. It was free from coarse-rooted forbs but supported small amounts of *Antennaria campestris* and *Aster multiflorus*. A few other grasses also occurred. These are so similar to little bluestem in root habit that the latter alone need be described. *Andropogon scoparius* developed a great mass of fine roots only 0.1 to 0.8 mm. in diameter. The roots were so abundant that they formed a dense sod. Many of them penetrated more or less vertically or obliquely downward, some reaching a depth of 4 feet. Others spread horizontally or obliquely in the surface soil. The surface foot was especially well occupied with dense masses of finely branched roots. They were also abundant in the second and third foot and even the fourth foot of soil was fairly well interspersed with fine roots, all of which were profusely branched (fig. 6).

The square meter selected for excavation on the big bluestem prairie contained, in addition to the dominant, a small amount of *Sorghastrum nutans* and a sprinkling of *Equisetum laevigatum*. The very abundant roots of *Andropogon furcatus* grew both vertically and obliquely downward, thoroughly occupying the soil and forming a dense sod. The larger roots varied from 0.5 to 3 mm. in diameter and a few reached a depth of 7 feet. The roots tapered so gradually that at 4 feet they were nearly as large as at the surface. All branched profusely even to near their tips (fig. 7). The roots of *Sorghastrum nutans* were similar in habit but did not penetrate so deeply.

The underground parts of *Equisetum laevigatum* were of special interest. They bulked large in amount, and have heretofore been undescribed. The tops originated from a much branched system of underground stems, which in the upper 2.5 feet of soil were mostly vertical or only slightly oblique. But from 2.5 to about 5.5 feet the stems grew horizontally or obliquely for long distances, giving rise



FIGS. 6, 7.—Fig. 6 (left), roots and tops of mature *Andropogon scoparius* from high prairie; fig. 7 (right), roots and tops of mature *A. furcatus* from low prairie. Scale in both figures is a meter stick.



to many branches which extended more or less vertically upward or downward. Some plants, however, were found that grew vertically downward to 7 feet with little or no branching. Usually the horizontal branching habit and an irregular downward course were characteristic features. Below 5.5 feet there were few horizontal branches, and even the vertically penetrating ones were much less abundant. The stemlike underground parts were of a steel blue or black color, distinctly jointed, and except for threadlike roots were like the parts above ground. The roots arose from the nodes or near them, often in groups of two or three but sometimes singly. A few were branched. None was found that exceeded 2.5 inches in length. In the deeper soil (usually below 5.5 feet) a type of branch arose from the horizontal stems that was apparently free from nodes, although the internal pattern, as observed with a hand lens, was similar to that of the stems. Moreover the short laterals arose irregularly all along the branch, sometimes at the rate of twelve per inch. While the underground stems were 6 to 7 mm. in diameter these roots were often only 4 to 5 mm. thick. Some were found that were thickly covered with a brown tomentum about 5 mm. long resembling root hairs. Numerous roots penetrated to a depth of 7.5 feet.

All roots were separated from the soil by a process of repeated washing, after the blocks of soil had been transported to the greenhouse. It was estimated that less than 1 per cent of the roots was lost, a negligible amount considering the variation in root distribution in the prairie.

### Methods of analysis

Soil samples for the volume-weight determinations were taken by means of special sampling equipment in use at the Nebraska Experiment Station. This equipment consists of a steel tube 4 inches in inside diameter and 2 feet long, fitted with a lining of easily removable brass cylinders, each 6 inches long. On the lower end of the steel tube is brazed a cutting ring whose inside diameter corresponds exactly with that of the brass linings. The tube is forced into the soil by means of a jack. Large augers are set in the ground about 30 inches apart, with a wooden span extending between them to serve as an anchor for the jack. A complete description of the apparatus

will be published soon (10). The field moisture contents and the volume-weights in table I were determined from 6-inch sections. The figures for the first and second 6-inch samples are the results of single determinations; those for all other depths are the average of the two 6-inch sections of each foot section. In each case the variation between the volume-weights of the two 6-inch sections was not more than a few hundredths.

The samples for the determination of organic matter and nitrogen and those for the determination of volume-weight were taken only a foot or two distant from the excavation made in securing the soil containing the roots. The samples for organic matter and nitrogen amounted in each case to several kilograms. They were dried and screened through a sieve of 4 meshes per inch, all roots and rhizomes being cut into pieces and mixed with the soil without loss. After thorough mixing of the large bulk sample, a 1 pint subsample was taken. The pint sample thus obtained was passed through a 2 mm. sieve, all roots being ground and retained as before. No gravel larger than 2 mm. in diameter was found in any of the samples, except an insignificant amount (less than 0.2 per cent) in the 2- to 4-foot depths of the Lancaster profile; these larger particles were discarded. The pint sample was again subsampled, about 100 gm. being taken and ground to pass through a 1 mm. sieve. This was used for the moisture equivalent and hygroscopic moisture determinations. Another 100 gm. portion was ground to pass through a 0.25 mm. sieve for the determinations of organic matter, nitrogen, and specific gravity.

The root materials were ground in a Wiley mill with a 2 mm. sieve. The organic matter of the roots was determined by ignition in an electric furnace. Nitrogen in the roots and soils was determined by the usual Gunning method (3). Organic matter in the soils was determined by a modification of the hydrogen peroxide method of ROBINSON (9). Duplicate determinations agreed within a few hundredths of 1 per cent. RUSSEL and ENGLE (12) have shown that the method is reliable and yields results agreeing closely with those obtained by multiplying organic carbon content by the conventional factor 1.724.

The hygroscopic coefficient was determined by the method of

ALWAY, KLINE, and McDOLE (1). The moisture equivalent was determined as described by RUSSEL and BURR (11). The specific gravity determinations were made by means of the pycnometer. The samples of both soils and roots for moisture determination were dried at 110° C.; the organic matter, nitrogen, and moisture percentages were calculated on the basis of oven-dry sample weights.

### Results

The moisture and volume relations of the soils are shown in table I. Mechanical analyses were not made, since the soil texture is clearly indicated by the hygroscopic coefficients.

TABLE I  
MOISTURE AND VOLUME RELATIONS IN THE SOILS

DEPTH (FEET OR INCHES)	HYGRO- SCOPIC COEFFI- CIENT (%)	FIELD MOISTURE CONTENT (F.M.C.) (%)	MOISTURE EQUIVA- LENT (%)	VOLUME- WEIGHT (GM./CC.)	SPECIFIC GRAVITY	PORE SPACE (%)	SPACE OCCUPIED BY WATER AT F.M.C. (%)	AIR SPACE AT F.M.C. (%)
Lancaster loam at Belmont prairie								
0-6"	11.0	30.4	35.4	1.12	2.59	56.8	34.1	22.7
6-12"	12.8	21.9	41.0	1.31	2.62	50.0	28.7	21.3
1-2'	12.6	14.9	37.8	1.41	2.63	46.4	21.0	25.4
2-3'	9.6	15.7	28.7	1.55	2.70	42.6	24.3	18.3
3-4'	5.6	13.5	14.8	1.64	2.68	38.8	22.1	16.7
Wabash clay loam at Everett prairie								
0-6"	18.2	20.7	41.9	1.05	2.60	59.6	21.7	37.9
6-12"	19.7	24.2	43.4	1.16	2.68	56.7	28.1	28.6
1-2'	15.6	23.2	36.1	1.10	2.61	57.9	25.5	32.4
2-3'	14.6	25.8	34.1	1.19	2.66	55.3	30.7	24.6
3-4'	15.8	29.2	36.1	1.20	2.66	54.9	35.0	19.9
4-5'	15.6	32.6	36.2	1.27	2.65	52.1	41.4	10.7
5-6'	14.2	31.4	35.5	1.24	2.66	53.4	38.9	14.5
6-7'	12.8	30.8	34.0	1.33	2.69	50.6	41.0	9.6

TEXTURE.—The figure 11.0 for the surface 6 inches of the Lancaster loam indicates a rather heavy loam texture; the greater hygroscopic coefficients of the 6- to 24-inch layer and the decreasing

figures below this depth agree well with the field observation of a clay horizon in the second foot and an increasing sandiness below this horizon.

In the Wabash clay loam the variations in hygroscopic coefficients may be taken to indicate corresponding variations in texture, the second 6 inches being the most clayey and the seventh foot the least clayey in the profile. Since this is an alluvial soil, no consistent variations can be found in the hygroscopic coefficients as the result of weathering processes in the profile, the texture of the deposited material having overshadowed any possible effects of weathering.

**MOISTURE EQUIVALENT.**—The moisture equivalents of the Lancaster soil samples are approximately three times the hygroscopic coefficients; those of the Wabash soils are approximately 2.5 times the hygroscopic coefficients. The moisture equivalent gives an approximate indication of the field moisture carrying capacity of the soil but exceeds it appreciably in soils of heavy texture. For example, the field moisture contents of the surface 6 inches of Lancaster loam, and of the lower 4 feet of the Wabash clay loam, were probably close to the field carrying capacity at the time of sampling, but they were appreciably lower than the moisture equivalents. Both soils had been dried considerably by the grass roots, as is indicated by their field moisture contents at the time of sampling, which are shown in table I.

**VOLUME-WEIGHT.**—The volume-weight of a soil is the weight of dry soil per unit volume. It is expressed in table I as grams per cubic centimeter. The volume-weight of the surface 6 inches of the Lancaster loam (1.12) may be considered a typical figure for virgin prairie soils of medium texture. It is low in comparison with that of the deeper horizons because of the high degree of granulation and the presence of a large amount of root material in the surface soil. The increasing volume-weight with increasing depth is correlated with decreasing granulation and also with increasing sandiness of texture. The figures 1.55 and 1.64 for the third and fourth foot sections indicate a high degree of compactness.

The volume-weight of the surface section of the Wabash soil is exceptionally low, owing to the highly granular structure of this horizon and the presence of a large volume of roots and rhizomes. The

volume-weight of the second foot is noticeably lower than that of the second 6 inches; it corresponds to that of a surface soil. This fact is in harmony with the field observation that the second-foot section is a buried surface soil. Below this level the volume-weight increased consistently with greater depth and less intensive root penetration and weathering.

**SPECIFIC GRAVITY.**—The specific gravity of the soil is influenced to some extent by the organic content. This fact is well illustrated by the figures given in table I. In the Lancaster loam the specific gravity increased with depth as the organic matter decreased. In the Wabash clay loam the specific gravity of the second-foot section was lower than that of the horizons above or below, corresponding with the higher organic content of this section. Below the 3-foot level the variations in specific gravity in this profile were of no significance.

**PORE SPACE.**—The pore space of the soil was calculated from the formula

$$P = 100 - \left( \frac{100 V}{S} \right),$$

in which P = per cent pore space, V = volume-weight, and S = specific gravity. The pore space decreases with depth as a general rule, being mainly an inverse function of the volume-weight. This relation is more obvious in the case of the Lancaster loam because of the sandier texture of the deeper horizons. Sandy soils as a rule have greater volume-weight and less pore space than heavy soils.

The percentage of the total soil volume occupied by water at any water content is readily calculated by multiplying the percentage of water on the dry weight basis by the volume-weight. This method has been used to calculate the percentage of the total volume of soil occupied by water at the field moisture content at the time of sampling. The difference between the total pore space and the space occupied by water is the percentage of air space in the soil at the water content in question. In figures 8 and 9 the air space is based upon the average water content of the soil during the season of 1932.

The data on air space at field moisture content in the Lancaster

loam indicate a well aerated profile throughout. The decrease in air space at the lower depths is due to the sandier texture and consequent smaller total pore space, while the decrease in air space in the deeper horizons of the Wabash soil is the result of higher water content. Even in the seventh foot, however, which was just above the ground-water level, the pore space of the soil was not entirely filled

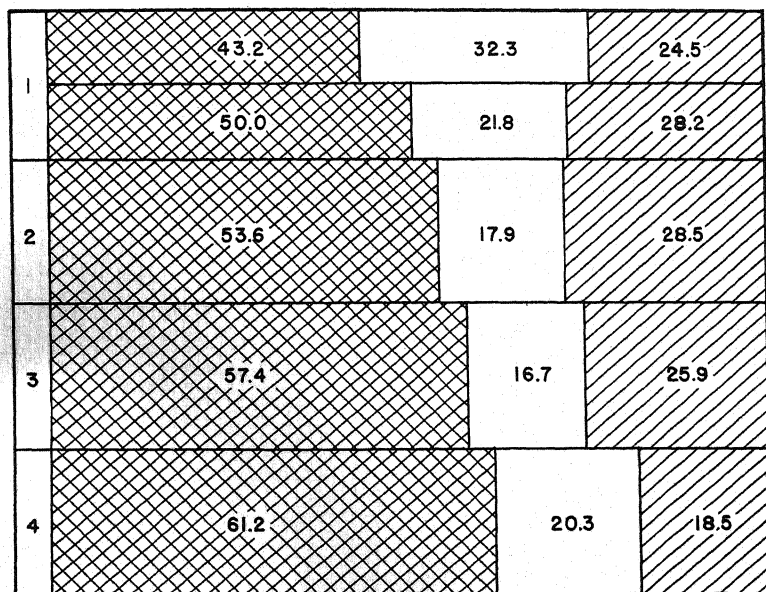


FIG. 8.—Diagram showing percentage of solid matter (cross hatch) and total pore space in first 4 feet of Lancaster loam soil covered with *Andropogon scoparius*. Portion of pore space occupied by water (single hatch) is based upon average water content during 1932; unhatched part is air space.

with water; about 10 per cent of the soil volume, or one-fifth of the total pore space, was occupied by air.

ORGANIC MATTER AND NITROGEN OF SOILS.—In table II are presented the soil organic matter and nitrogen relations in the two profiles. The nitrogen in the soil organic matter (fifth column) is the reciprocal of the ratio of organic matter to nitrogen (fourth column) and is added for the convenience of those who are accustomed to think of the relation between nitrogen and organic matter on a percentage basis rather than as a ratio. The carbon-nitrogen

ratio (sixth column) has been calculated from the organic matter-nitrogen ratio by dividing by the conventional factor 1.724. It is

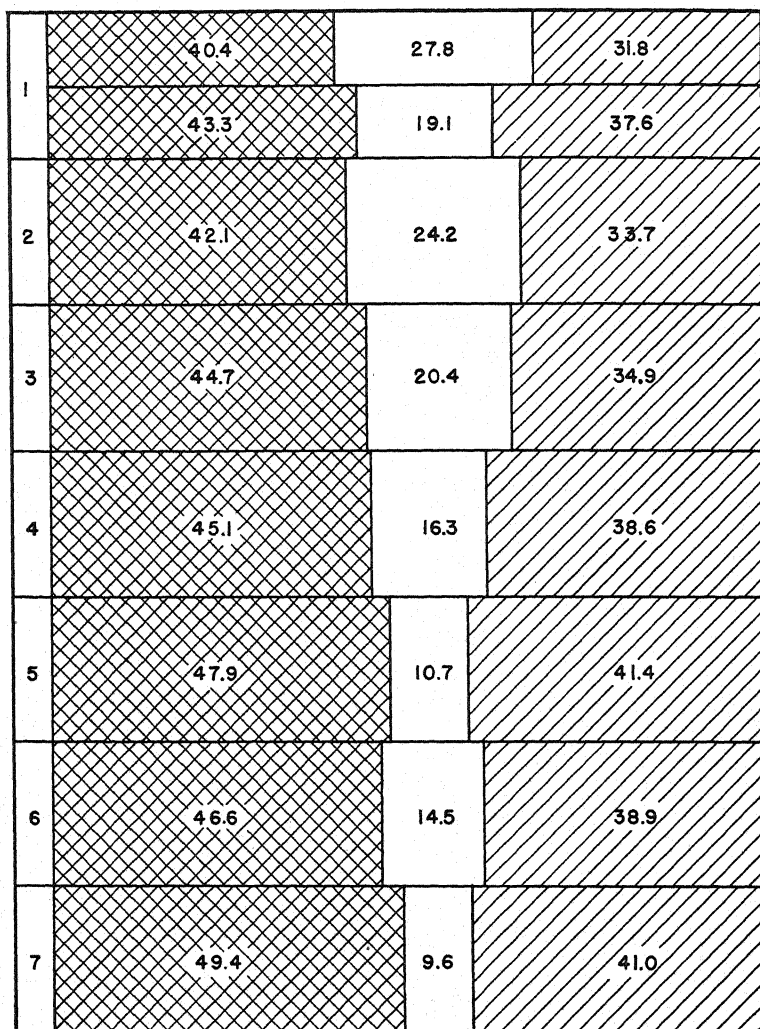


FIG. 9.—Diagram showing percentage of solid matter, air space, and water in the first 7 feet of Wabash clay loam soil covered with *Andropogon furcatus*. Legend and conditions as in figure 8.

presented for the purpose of comparison with other carbon-nitrogen ratios in the literature.

The weight of soil per square meter (seventh column) has been calculated from the volume-weight. The first two figures for each soil are for 6-inch sections, while the other figures correspond to 1-foot sections. Since the volume of one square meter to the depth of 6 inches is 152,400 cc., the weight of soil in grams per square meter for the 6-inch sections is obtained by multiplying this figure by the volume-weight; for the foot sections, weight per square meter equals

TABLE II  
ORGANIC MATTER AND NITROGEN RELATIONS IN THE SOILS

DEPTH (FEET OR INCHES)	ORGANIC MATTER (%)	NITROGEN (%)	RATIO OM/N	NITROGEN IN SOIL OM (%)	RATIO C/N	WT. OF SOIL PER SQ.M. IN KG.	ORGANIC MATTER (GM./SQ. M.)	NITROGEN (GM./SQ. M.)
Lancaster loam at Belmont prairie								
0-6"	4.50	0.224	20.0	5.0	11.6	171	7670	382
6-12"	3.24	.167	19.4	5.2	11.3	200	6480	334
1-2'	1.59	.093	17.1	5.8	9.9	430	6840	400
2-3'	0.43	.043	10.0	10.0	5.8	472	2030	203
3-4'	0.20	.024	8.3	12.0	4.8	500	1000	120
Wabash clay loam at Everett prairie								
0-6"	4.92	.262	18.8	5.3	10.9	160	7880	420
6-12"	2.24	.138	16.2	6.2	9.4	177	3960	244
1-2'	2.77	.167	16.6	6.0	9.6	335	9280	560
2-3'	1.75	.114	15.3	6.5	8.9	363	6340	414
3-4'	1.24	.088	14.1	7.1	8.2	366	4540	322
4-5'	1.44	.078	18.5	5.4	10.7	387	5580	302
5-6'	1.22	.074	16.5	6.1	9.6	378	4600	280
6-7'	1.02	0.061	16.7	6.0	9.7	405	4140	248

volume-weight  $\times 152,400 \times 2$ . The weights given in the table are expressed in kilograms. These figures were used in converting the percentage of organic matter and nitrogen in the soil to grams per square meter in order to obtain the weight of soil organic matter and nitrogen in the volume of soil from which the roots were taken, as shown in the last two columns of table II.

The organic matter and nitrogen data for the Lancaster loam show the profile characteristics usually found in normal grassland soils. According to the extensive work of ALWAY and McDOLLE (2) and of



RUSSEL and McRUER (13) in grassland soils, the nitrogen and organic matter decrease regularly with depth, the bulk of the organic matter being confined to the surface foot. In the first and second 6-inch sections, the organic matter and nitrogen contents are in agreement with those found by these investigators for soils of this texture in this part of the state; in the lower sections the organic content decreases more rapidly as a result of their sandy texture. The organic matter-nitrogen ratios throughout the profile are typical of the upland soils of this region.

The percentage of organic matter and nitrogen in the second foot of the Wabash silt loam is considerably higher than that in the 6-inch section just above it, correlating perfectly with the field observation that this layer shows the characteristics of a well developed surface soil. In virgin prairie soils, a higher nitrogen content is never found in a lower layer than in layers above, except in cases of buried soil profiles or where silting by wind or water has occurred comparatively recently (13). The ratio of organic matter to nitrogen shows a tendency toward that of the surface soil, for although the ratio 16.6 is perhaps not significantly higher than the ratio 16.2 in the second 6 inches, yet if the profile showed the characteristics of a mature soil, the ratio in the second foot should be significantly lower than 16.2. This is illustrated by the organic matter-nitrogen ratios of the Lancaster loam.

The relatively high values for the organic matter-nitrogen ratios of the lower 3 feet of the profile are believed to indicate that the 4-foot level also was at one time a surface soil. The slightly higher organic content of the fifth foot in comparison with the fourth foot is in agreement with this conclusion. In the field a rather abrupt change in the appearance and properties of the soil was observed at approximately the 4-foot level.

It seems remarkable that the soil organic matter should retain its characteristics after being buried and subjected to decomposition and weathering for a period that was undoubtedly several hundred years in duration, but the data in table II indicate that such is the case.

DRY WEIGHT, ORGANIC MATTER, AND NITROGEN OF ROOTS.—The dry weight, organic matter, and nitrogen of the roots recovered from

each section of the Lancaster loam are shown in table III. Nearly 60 per cent of the total root system was found in the upper 6 inches of soil. This, however, includes the rhizomes.

The high organic matter of the roots and short rhizomes of the little bluestem in the surface foot of soil is probably due to the presence of stored food reserves. The lower figures in the second and third foot may be due to decortication of the older roots in these sections, while the increase in the fourth foot may result from this portion of the root system containing the younger and smaller root-ends which often have a relatively larger proportion of living tissues.

TABLE III  
DRY WEIGHT, ORGANIC MATTER, AND NITROGEN IN ROOTS  
FROM ONE SQUARE METER OF LANCASTER LOAM

DEPTH (FEET OR INCHES)	DRY WEIGHT (GM.)	ORGANIC MATTER (%)	NITROGEN (%)	ORGANIC MATTER (GM.)	NITROGEN (GM.)
0-6"	741.0*	90.8	0.828	657	6.13
6-12"	198.0	91.7	0.699	182	1.39
1-2'	193.0	83.1	0.656	160	1.26
2-3'	85.5	86.6	0.730	74	0.62
3-4'	12.0	91.4	0.800	11	0.10

\* This includes also the rhizomes of the grasses all of which occurred in the surface 6 inches of soil.

In the Wabash profile it was possible to separate the grass roots from the non-grasses and in the surface layer to separate the grass roots from the rhizomes. The dry weight, organic matter, and nitrogen in these roots and rhizomes are shown in table IV. In the fifth foot and deeper, the quantity of grass roots alone was too small for analysis, hence the grasses and non-grasses were mixed. The organic matter in the non-grasses above the fifth foot varied from 78 to 88 per cent, with no consistent relation to depth. The high organic matter and nitrogen content of the rhizomes, 94 and 0.73 per cent respectively, may be attributed to their store of food reserves. The organic matter of the grass roots in the surface 6 inches, 74 per cent, was found to be considerably lower than in the deeper horizons, where the figure varied from 81 to 91 per cent. This may be due to the high degree of decortication of the older roots in the surface layer and to the leaching of soluble organic matter from the dead and

partly decayed roots. Unfortunately the roots and rhizomes of the little bluestem in the Lancaster soil were not separated, and the

TABLE IV  
DRY WEIGHT, ORGANIC MATTER, AND NITROGEN IN ROOTS  
FROM ONE SQUARE METER OF WABASH CLAY LOAM

DEPTH (FEET OR INCHES)	KIND OF ROOTS, ETC.	DRY WEIGHT (GM.)	ORGANIC MATTER (%)	NITROGEN (%)	ORGANIC MATTER (GM.)	NITROGEN (GM.)
0-6"	Non-grasses*	23.2	84.3	0.594	19.6	0.138
	Grasses	726	74.3	.515	539	3.74
	Rhizomes of grasses	292	94.0	.728	274	2.12
	Total.....	1041	.....	.....	833	6.00
6-12"	Non-grasses	12.4	86.0	.372	10.6	.046
	Grasses	168	84.6	.500	142	.838
	Total.....	180	.....	.....	153	.884
1-2'	Non-grasses	34.0	78.3	.340	26.6	0.116
	Grasses	179	81.0	.532	145	.954
	Total.....	213	.....	.....	172	1.07
2-3'	Non-grasses	43.6	86.3	.301	37.6	.132
	Grasses	80.2	87.8	.514	70.8	.412
	Total.....	124	.....	.....	108	0.544
3-4'	Non-grasses	22.0	88.0	.305	19.4	.066
	Grasses	42.2	90.8	.456	38.2	.194
	Total.....	64.2	.....	.....	57.6	.260
4-5'	Non-grasses	42.6	.....	.....	.....	.....
	Grasses	14.0	.....	.....	.....	.....
	Total.....	56.6	87.4	0.332	49.4	.188
5-6'	Non-grasses	11.4	.....	.....	.....	.....
	Grasses	2.6	.....	.....	.....	.....
	Total.....	14.0	90.2	.342	12.6	0.048
6-7'	Non-grasses	11.4	.....	.....	.....	.....
	Grasses	0.6	.....	.....	.....	.....
	Total.....	12.0	78.1	0.409	9.4	0.049

\* Non-grasses are very largely *Equisetum laevigatum*.

separate composition of each of these structures alone is not known.  
The data in table V are taken from the preceding tables. They

permit several conclusions to be drawn concerning the relationships between the depth in the soil profile, the quantity of organic matter present, the quantity of roots, and the type of decomposition of the dead root material. It should be borne in mind that the "soil organic matter" of the tables includes that of the root material. This is the case because in the routine preparation of soil samples for analysis it is not practicable to remove all of the root material from the soil,

TABLE V  
ORGANIC MATTER AND NITROGEN RELATIONS BETWEEN SOILS AND ROOTS

DEPTH (FEET OR INCHES)	ORGANIC MATTER (GM.)		RATIO SOIL OM ROOT OM	NITROGEN (GM.)		RATIO SOIL N ROOT N
	IN SOILS	IN ROOTS		IN SOILS	IN ROOTS	
Lancaster loam at Belmont prairie						
0-6"	7670	657	11.7	382	6.13	62
6-12"	6480	182	35.7	334	1.39	241
1-2'	6840	160	42.7	400	1.26	316
2-3'	2030	74.0	27.4	203	0.62	325
3-4'	1000	11.0	90.9	120	0.10	1250
Wabash clay loam at Everett prairie						
0-6"	7880	833	9.46	420	6.00	70
6-12"	3960	152	26.0	244	0.88	276
1-2'	9280	172	54.0	560	1.07	523
2-3'	6340	108	58.5	414	0.544	765
3-4'	4540	57.6	78.9	322	.260	1210
4-5'	5580	49.4	113	302	.188	1590
5-6'	4600	12.6	365	280	.048	5880
6-7'	4140	9.4	440	248	.049	5020

nor is it possible to remove a part of this material without introducing the factor of personal judgment as to how much of the coarse roots should be sifted out and how much should be retained. The only simple method of avoiding the personal factor is to retain all of the root material, and this is the usual procedure in obtaining and preparing soil samples for analysis.

In the surface soil, according to table V, approximately one-tenth of the "soil organic matter" consists of plant roots and rhizomes, so that whether or not they are included as a part of the soil sample is a

matter of some importance. In the deeper layers the roots are of relatively less importance as a part of the organic matter of the soil. Figure 10 shows graphically the relations between the depth in the soil and the ratio of soil organic matter to the organic matter of the roots throughout each of the two profiles, and illustrates the fact that with increasing depth the mass of the roots becomes decreasingly important in proportion to that of the soil organic matter. The ratio of soil organic matter to the organic matter of the roots is roughly a linear function of the depth in the Lancaster profile. This is also true in the Wabash profile to the depth of 5 feet, but in the sixth and seventh foot the quantity of soil organic matter is far out of proportion to the quantity of roots. This is probably due to the excessive water content and deficient aeration which tend to inhibit the complete decomposition of organic matter and preserve it in the soil in the partly decomposed or humified state. The same statements apply to the relation between the depth in the profile and the ratio of soil nitrogen to root nitrogen which is not shown graphically because of its similarity to the organic matter relation.

The point corresponding to the 3-foot depth in the Lancaster soil deviates widely from the other points in this profile with respect to both organic matter and nitrogen ratios. This is believed to be due to the fact that the third foot is a sandy horizon, very permeable to grass roots, and capable of holding sufficient water and air for the rapid decomposition of dead roots. These conditions might be expected to result in the presence of a relatively large mass of living roots and a small amount of dead residues.

The quantity of roots found at any level in the soil depends to some extent on the fertility and physical properties of the soil, but is principally dependent upon the nature of the plant, unless the soil departs widely in parts of the profile from the conditions of texture, structure, fertility, and moisture usually found in the grassland soils of this region. In the case of the Wabash soil, it is evident that the organic matter of the soil has had no great influence on root development, for the 6- to 12-inch level contained 2.24 per cent of organic matter and 0.10 per cent of root material, while the 1- to 2-foot section contained 2.77 per cent of organic matter and only 0.06 per cent of root material. The lower layer of soil, relatively one-fifth

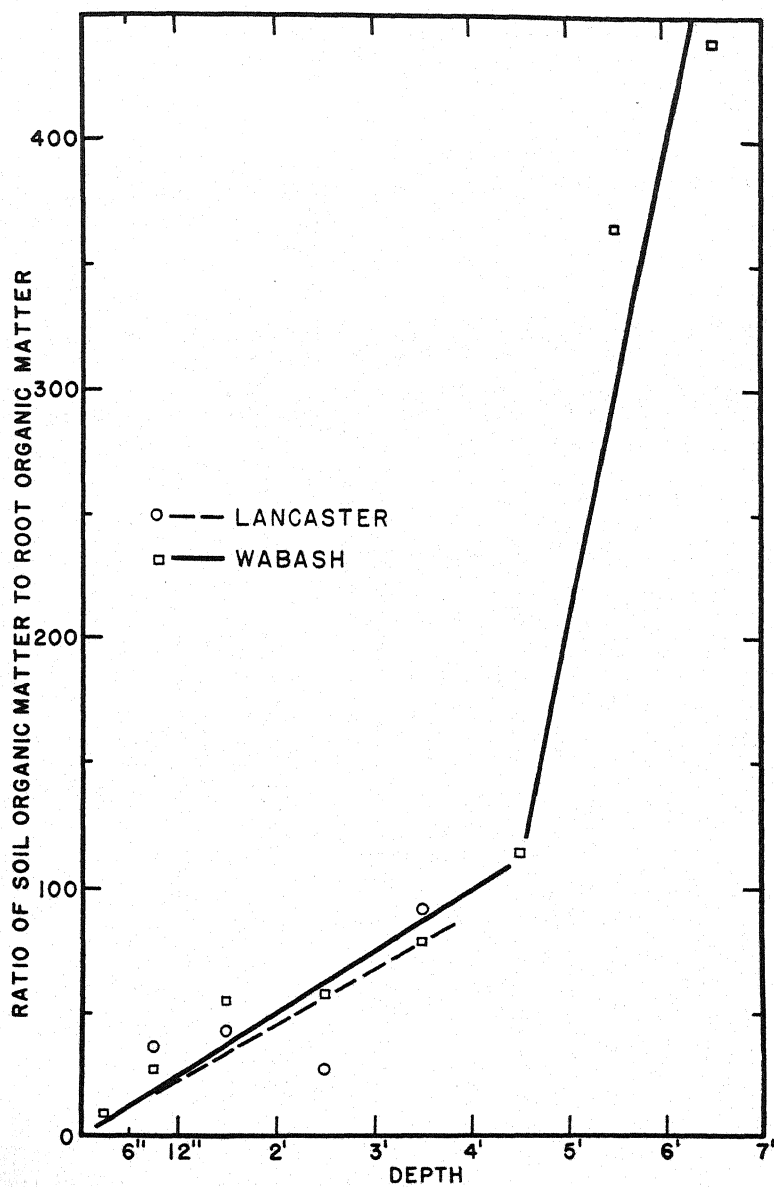


FIG. 10.—Relation between depth in profile and ratio of soil organic matter to root organic matter.

richer in total organic matter, was relatively two-fifths poorer in root material.

The relation between the amount of organic matter in the soil and that in the roots is shown graphically in figure 11. The data for the organic matter of both soil and roots in the first and second 6-inch sections have been multiplied by two in order to place all the data on

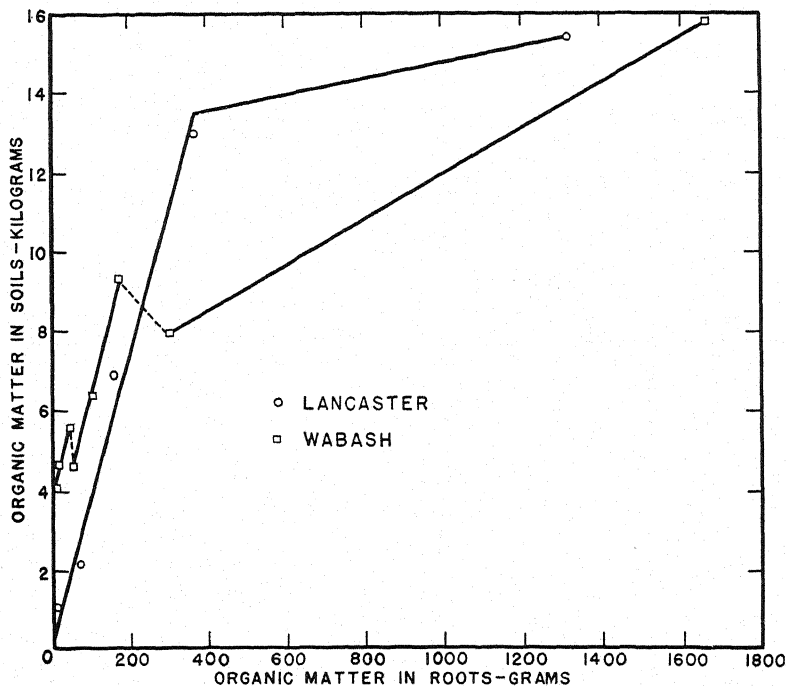


FIG. 11.—Relation of organic matter in roots to organic matter in soils.

the foot-section basis. The graph for the Lancaster soil is approximately a straight-line function except in the surface 6 inches, where the large mass of roots far exceeds the proportionate amount of soil organic matter. In the Wabash soil, however, there are breaks in the curve, corresponding to the positions of the buried surface soils in the profile. Between these points of discontinuity the slope of the curve is approximately the same as for the Lancaster soil. Although it is not advisable to make a positive statement based on the study

of but two profiles, these results suggest a possible constant relation, except in the surface layer of soil, between root development and soil organic matter.

### Discussion

The nitrogen content of the soil organic matter is dependent on the climatic and soil conditions, and the character and amount of plant growth. Under virgin conditions the plant growth ultimately reaches an equilibrium which is the result of the interaction of the climatic conditions, the soil conditions, and the plant growth itself. The nitrogen content of the soil organic matter, which may also be expressed in the form of the carbon-nitrogen ratio or the organic matter-nitrogen ratio, is thus the result of an equilibrium between the accumulation of plant residues and their decomposition by molds and bacteria in the soil.

WAKSMAN (16) has explained the relatively constant carbon-nitrogen ratio of the soil organic matter and its significance as follows:

"It could not be otherwise; were the carbon content too large in relation to the nitrogen, the soil would not be in a condition to support plant growth, as long as this excess lasted; the microorganisms using the carbon as a source of energy would assimilate every trace of available nitrogen that would otherwise be made available for the growth of higher plants. Were the nitrogen content too large, ammonia would be rapidly liberated and then transformed to nitrates and either leached out or assimilated by higher plants."

WAKSMAN has estimated the carbon-nitrogen ratio of fungus mycelia to be about 9 or 10, and that of bacteria about 4 or 5. Undecomposed plant material has a ratio varying from 20 in legumes to 80 or more in wheat straw. The carbon-nitrogen ratio of the soil may thus be considered an index of the relative amounts of undecomposed plant residues, fungi, and bacteria present. SIEVERS and HOLTZ (14) have called attention to the fact that virgin soils have a wide ratio and that under cultivation the ratio tends to become narrower, as illustrated by the following table:

Virgin soil. . . . .	13.1	12.5	11.3	14.0
Cropped 40 years. . . . .	9.4	11.3	11.0	12.0



The carbon-nitrogen ratio in cultivated or fallowed soils, according to these investigators, tends to approach 9 as a minimum value. This is due mainly to the smaller supply of decomposable material returned to the soil each year, and probably to some extent to the more favorable conditions for decay in the cultivated soil.

ALWAY and McDOLLE (2) have reported the carbon-nitrogen ratios in successive foot-sections of virgin soil at Lincoln to be 12.0, 10.2, 9.5, 5.9, 6.0, and 5.4. In table II are presented the organic matter-nitrogen ratios and the corresponding carbon-nitrogen ratios of the several horizons of the Lancaster loam. The ratios decrease rapidly with increasing depth, and in this respect may be considered typical of normal upland prairie soil profiles. The narrower ratios at greater depths may be explained by the presence of relatively small quantities of root residues in proportion to relatively large quantities of the decomposition products of fungal and bacterial activity. BROWN and BENTON (5) have made a comprehensive study of the distribution of microorganisms in the prairie soils of Iowa. They have shown that the bacteria and actinomyces as a rule far outnumber the molds throughout the profile. The predominance of the bacteria and actinomyces tends to keep the carbon-nitrogen ratio low in those parts of the soil where the organic residues that form the food supply of the organisms are small in amount.

The organic matter-nitrogen ratios throughout the Wabash profile, shown in table II, are essentially those of surface soil. They appear to indicate that where the surface soil has accumulated organic matter and then been buried under fresh sediments, the organic matter so buried retains the characteristics of surface material for a long time. The explanation for such behavior is difficult to find. It may be tentatively suggested, without definite experimental proof applied to the conditions existing in these soils, that the surface type of organic matter contains a large proportion of lignins and lignin-like materials, non-nitrogenous substances which are resistant to decay. According to WAKSMAN and IYER (17), the lignins will combine with proteins, forming complexes that are highly resistant to decomposition, and which may be expected to persist in the soil unchanged for a long time. Decomposition would be especially slow in soils nearly saturated with water, as in the lower layers of the

Wabash profile. Additional evidence to support this theory is found in the fact that these layers contain relatively large amounts of organic matter in comparison with the corresponding sections of upland soils. The Wabash profile contains 1.24, 1.44, and 1.22 per cent of organic matter in the fourth, fifth, and sixth foot sections respectively, while the corresponding sections of loess soil at Lincoln were reported by ALWAY and McDOLLE (2) to contain 0.60, 0.43, and 0.40 per cent of organic matter.

### Summary

1. A typical square meter of vegetation was selected in the upland, *Andropogon scoparius* prairie on Lancaster loam soil, near Lincoln, Nebraska, and another in the lowland, *A. furcatus* prairie on the Wabash clay loam soil of the floodplain of the Missouri River.
2. The surface soil was removed in 6-inch layers and the deeper soil in foot sections to the depth of root penetration; the roots and rhizomes were carefully removed by washing, and their dry weights, nitrogen contents, and organic contents determined.
3. Water content of soil and atmospheric factors affecting plant development were measured and rate of growth of the vegetation was determined. Root habits of the plants excavated were noted.
4. The soils varied so uniformly in texture, structure, and fertility with depth, that (except in buried surface layers) root distribution was of the usual type found for these species over a wide range of prairie soils.
5. In the Lancaster loam 60 per cent of the underground parts of the dominant grass was found in the surface 6 inches; the remainder of the root system was distributed to a depth of 4 feet. In the Wabash clay loam 68 per cent of the underground parts (60 per cent excluding rhizomes) was found in the surface 6 inches; the remainder of the root system extended to the depth of 7 feet and nearly to the water table.
6. The hygroscopic coefficient and moisture equivalent of each section of the soil profile were determined as indexes of the texture. The volume-weight of the soil in the undisturbed field condition was determined. From the volume-weight, specific gravity, and field moisture content at the time of sampling, it was possible to calculate

the pore space, space occupied by water, and air space in each section of the soil.

7. Volume-weight in the Lancaster loam increased gradually from 1.12 in the surface 6 inches, where an abundance of roots, rhizomes, and dead organic matter filled the soil, to 1.64 in the fourth foot, where both living roots and dead organic matter were relatively sparse.

8. Volume-weight in the Wabash clay loam increased gradually (except in buried surface soils) from 1.05 in the first 6 inches to 1.33 in the seventh foot. This was accompanied by a gradual decrease in both living root materials and dead organic matter.

9. Pore space in the surface 6 inches of Lancaster loam constituted 57 per cent of the volume of the soil. On an average 25 per cent was occupied by water and 32 per cent by air. It decreased with depth to 39 per cent at 4 feet, where 19 per cent was occupied by water and 20 per cent by air.

10. Pore space in the surface 6 inches of Wabash clay loam occupied 60 per cent of the soil volume. On an average 32 per cent was filled with water and 28 per cent with air. It decreased regularly with depth (except for buried surface layers) to about 51 per cent in the seventh foot, where water occupied 41 per cent and air only 10 per cent.

11. The organic matter and nitrogen content of the roots in the upland soil were somewhat higher than in the lowland, especially in the surface 6 inches.

12. The composition of the rhizomes in the lowland was determined separately; their organic matter and nitrogen content were greater than in the roots, owing to the storage of food reserves.

13. The percentage of organic matter and nitrogen in each layer of the soil was determined, and the weight of each per square meter of soil calculated.

14. The ratio of soil organic matter to nitrogen varied in the Lancaster loam from 20 in the surface soil to 8.3 in the fourth foot, a variation typical of upland prairie soils.

15. The ratio of organic matter to nitrogen in the Wabash clay loam showed variations corresponding to the variations in the organic content at different depths. This indicates that the strata

of high organic content had accumulated a surface type of organic matter during intervals in the silting process by which the present soil has been built up.

16. Except in the surface 6 inches of soil, there is an approximately linear relation between the amount of root material and the amount of soil organic matter in the various soil horizons.

17. In the surface soil the presence of a large amount of living rhizome and root material and the favorable conditions for the decomposition of dead organic matter increase the proportion of roots and rhizomes to soil organic matter.

18. Roots and rhizomes constitute about one-tenth of the total organic matter in the surface 6 inches of soil; in the deeper sections the proportion decreases gradually from 3 to 4 per cent in the second 6 inches to 1 per cent in the fourth foot of the Lancaster soil, and 0.25 per cent in the seventh foot of the Wabash soil.

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# ALTERNATION OF GENERATIONS IN *ECTOCARPUS SILICULOSUS*\*

GEORGE F. PAPENFUSS

(WITH PLATES VI, VII AND THIRTEEN FIGURES)

## Introduction

After the publication of the paper by BERTHOLD (2) in 1881, the plurilocular sporangia of *Ectocarpus siliculosus* Dillw. were regarded as gametangia and their zooids as gametes. Many investigators (21-24, 9-12, and others) found, however, that the zooids from the plurilocular sporangia of this species do not consistently function as gametes but more frequently act as zoospores. In order to explain this asexual behavior, the theory was generally advanced that the gametes had lost their sexual power and germinated parthenogenetically. The cytological work of KNIGHT (7) has proved this theory to be incorrect, for it has shown that there are two kinds of plurilocular sporangia in *E. siliculosus*: one type occurring on haploid plants and forming gametes and the other type occurring on diploid plants and giving rise to diploid zoospores which germinate into other diploid plants.

KUCKUCK (9) and REINHARDT (20) found that the zooids from the unilocular sporangium of *E. siliculosus* are zoospores while KNIGHT (7) believes that they function as gametes at Port Erin. The cytological studies of KNIGHT showed all the plants in this locality to be diploid. No reduction divisions occur in the plurilocular sporangium, and the zooids from this organ are diploid zoospores which germinate directly into other diploid plants. In the unilocular sporangium, on the contrary, the first division of the nucleus is a reduction division and the zooids formed are haploid. These zooids conjugate to form zygotes which develop into diploid plants. At Port Erin, therefore, no alternation of generations occurs; all the plants are diploid.

KNIGHT (7) studied *Ectocarpus* at Naples also, and confirmed the observations of BERTHOLD (2), OLTMANN (18), and HARTMANN (5) that the zooids from the plurilocular sporangium are gametes in this

\* Botanical contribution from The Johns Hopkins University, no. 127.

locality. The cytological work of KNIGHT showed, moreover, that the plants at Naples are haploid. Diploid plants are reported to be absent here. Since no reduction division occurs at the germination of the zygote in any brown alga thus far investigated, it is very probable that the zygotes at Naples develop into diploid plants.

KYLIN (13) in 1918 predicted the discovery of an alternation of generations in this species, and the cytological work of KNIGHT (7) suggests that it may occur in certain localities. It thus becomes important to know what rôle is played by the zoids from the unilocular sporangia on diploid plants, in all places where, as at Naples, the zoids from the plurilocular sporangia of haploid plants serve as gametes. Do the zoids from the unilocular sporangia in such localities act as gametes, as at Port Erin, or are they zoospores? It would be of great interest if they should function as gametes for it would show that this species has an alternation of generations and that both generations are capable of forming gametes, a unique condition among plants. Furthermore, may unilocular sporangia occur on haploid plants; and, if so, what is the function of the zoids?

It was with these problems in mind that the writer took up this study. The preliminary observations were made on plants found floating near Annapolis in the Chesapeake Bay during April, 1930. These plants bore only plurilocular sporangia and the zoids were zoospores. During the late autumn of 1930, material was collected at Cold Spring Harbor, Long Island, New York. This material also contained only plurilocular sporangia and the zoids were again asexual.

In order to obtain more abundant material than was found in the upper Chesapeake Bay, and to secure if possible plants with unilocular sporangia, this study was continued at Woods Hole, Massachusetts, over a period of three years. A brief preliminary account of the results obtained has been published (19). The present paper gives a more complete account of the functions of the different kinds of zoids, of the cytology of plants from the sea, and of the results obtained from cultural studies.<sup>2</sup>

<sup>2</sup> The following two papers on the life history of *E. siliculosus* appeared after this paper had been sent to press: FÖYV, B., Über den Lebenszyklus einiger Braunalgen. Bergens Museums Årbok. Nat. 2:1-9. 1934; SCHUSSNIG, B., and KOTHBAUER, E., Der Phasenwechsel von *Ectocarpus siliculosus*. Österr. Bot. Zeitsch. 83:81-97. 1934.

### Observations on living marine plants

#### A. FUNCTION OF ZOIDS OF ASEYUAL PLANTS

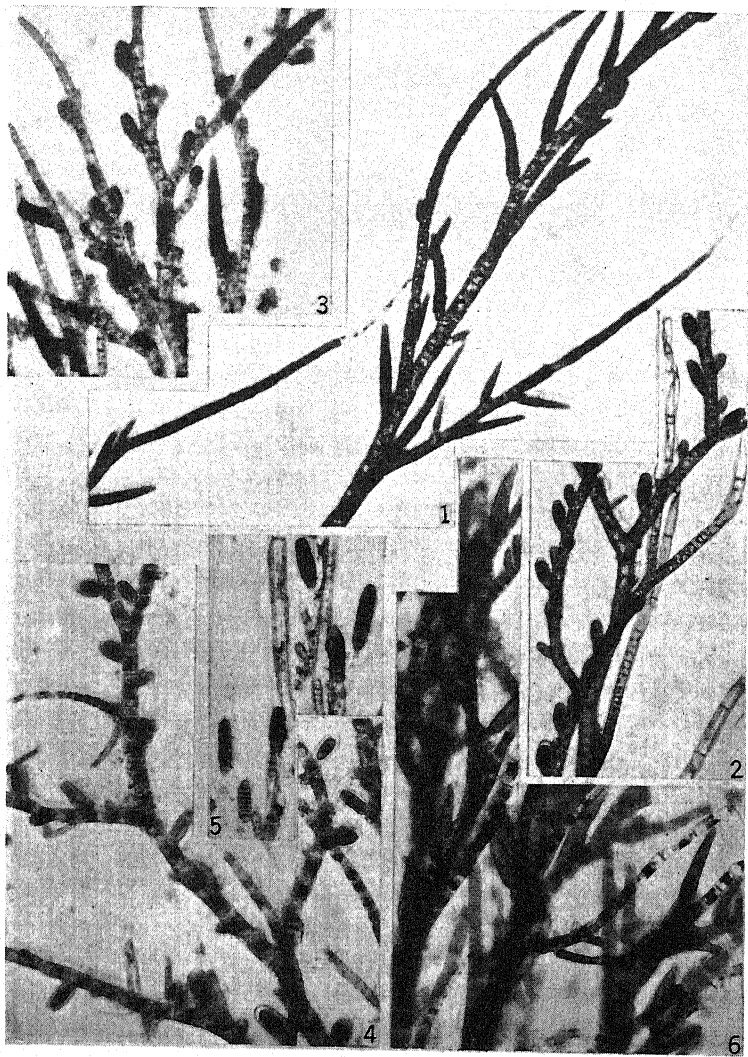
All the observations on the function of the different kinds of zoids were made by placing pieces of *Ectocarpus* in a hanging drop and studying the behavior of the zoids liberated. A small drop of water was allowed to flow under the edge of the cover. This prevents the cover from sliding off the depression in the slide and also minimizes evaporation from the suspended drop. If it was necessary to keep a slide longer than five or six hours, the slide was placed in a damp chamber and the material in the suspended drop transferred to a fresh drop at least once a day. The water was changed usually in the morning since this is the time that liberation of zoids commonly occurs and a change of water often causes immediate liberation.

*Ectocarpus siliculosus* was first collected at Woods Hole during the early part of June, 1931; and was found on *Zostera* growing at Grassy Island. These plants bore only plurilocular sporangia (fig. 1) and the zoids were zoospores. Unilocular sporangia (fig. 2) were found during the third week of June on plants which were floating or which were caught on the ropes of lobster pots between Nobska Point and Nonamesset Island. The liberation of zoids from these sporangia was awaited with keen interest, for it was expected, in the light of KNIGHT'S (7) work, that the zoids would function as gametes. Contrary to such expectation, conjugation never occurred among the zoids from the unilocular sporangia, and the early germination of practically every individual zoid showed that they are zoospores.

Occasionally certain plants from the Nobska-Nonamesset region bore plurilocular as well as unilocular sporangia. The zoids from the plurilocular sporangia of such plants were also uniformly asexual. *E. siliculosus*, bearing an abundance of both unilocular and plurilocular sporangia (fig. 3) and growing as an epiphyte on *Chorda filum*, was collected later in the season at Nobska, Grassy Island, and Pine Island. During mid-summer about 50 per cent of the unilocular sporangia were parasitized by a chytrideaceous fungus; but this did not interfere with the study since many uninfected sporangia were to be had.

As plants of *E. confervoides* were found which also bore both unilocular and plurilocular sporangia simultaneously, a number of





FIGS. 1-6.—Unretouched photomicrographs showing: Fig. 1, branch of diploid plant of *E. siliculosus* bearing the plurilocular sporangia by which this species is characterized taxonomically. Material fixed in formalin acetic alcohol. Fig. 2, branch of diploid plant of same bearing unilocular sporangia only. Chromo-acetic-osmic acid material. Fig. 3, branch of diploid plant of same bearing plurilocular and unilocular sporangia on same individual. Formalin acetic alcohol material. Fig. 4, branch of *E. confervoides* with unilocular sporangia. Note that majority of unilocular sporangia are sessile in *E. confervoides* while they are usually stalked in *E. siliculosus*. Formalin material. Fig. 5, branch of *E. confervoides* bearing plurilocular sporangia. Formalin material. Fig. 6, branch of haploid plant of *E. siliculosus* bearing plurilocular organs which function as gametangia. Note that these are smaller than the plurilocular sporangia of the diploid asexual plants as shown in figs. 1, 3. Formalin material. All  $\times 100$ .

observations were made on the behavior of the zoids of this species. The results obtained were the same as those for *E. siliculosus*, for the zoids from both kinds of sporangia were zoospores which germinated directly. Unilocular and plurilocular sporangia occurring on separate plants of *E. confervoides* are shown in figures 4 and 5.

#### B. FUNCTION OF ZOIDS OF SEXUAL PLANTS

In July of 1931 *Ectocarpus siliculosus*, growing on *Chordaria flagelliformis*, was collected on the west end of Penikese Island, about 20 miles southwest of Woods Hole. These plants of *Ectocarpus* bore only plurilocular sporangia. As the material was comparatively free from diatoms, it was used for cytological work and fixed hourly during the next 24 hours. The morning of the third day after collection offered the first opportunity for studying the living plants. Although the material appeared to be in good condition, no liberation of zoids occurred and the plants finally died.

Cytological studies made the following winter showed that the plants from Penikese were haploid while the plants collected at Woods Hole proper, judging from the chromosome number in the plurilocular sporangia, were diploid. The cytology of the unilocular sporangium remained unsettled at the time.

Since no conjugation occurred between the presumably haploid zoids from the unilocular sporangia, it seemed clear that if conjugation occurred in the Woods Hole region, it must be between the zoids of the haploid plants at Penikese.

In order to determine whether the haploid plants were sexual, the writer returned to Woods Hole in August, 1932, and visited Penikese in early September. *E. siliculosus* was again found on *Chordaria* at the same locality as in the previous summer. In addition, other plants were obtained also on *Chorda* which grew mixed with the *Chordaria*. This collection presented two problems for solution: (1) Do the plants on *Chordaria* produce gametes? (2) Are the plants on *Chorda* from this locality asexual and do they bear both kinds of sporangia simultaneously, similar to those on *Chorda* at Woods Hole? If these two questions could be answered in the affirmative, it would show that an alternation of generations occurs in the life cycle of *E. siliculosus* at Penikese.

A microscopic examination showed that the plants on *Chorda* were similar to those occurring on this host at Woods Hole, in that they bore both kinds of sporangia, while the plants on *Chordaria* bore only plurilocular sporangia (fig. 6). The plants on *Chorda* liberated zoids freely the following morning and the zoids from both kinds of sporangia proved to be zoospores. It could not be determined whether the plants on *Chordaria* were sexual as no liberation of zoids occurred and the plants finally died. These plants at first appeared perfectly healthy and it was difficult to understand why no zoids were liberated, since the plants on *Chorda* which were collected at the same time and kept in similar running water aquaria liberated zoids freely.

It appeared probable that liberation of zoids might occur if the plants growing on *Chordaria* were taken directly from the ocean. On September 10, therefore, plants of *E. siliculosus* growing on *Chordaria* and *Chorda* respectively were collected at Penikese. Separate hanging-drop cultures were made of plants from each host. Pieces from several plants were placed in each culture. No liberation of zoids occurred that day but the following morning at daybreak liberation of zoids occurred in practically every drop and many conjugations were observed. The zygotes (fig. 14) were formed, however, only in the hanging drops containing plants growing on *Chordaria*. Although hundreds of zoids from both kinds of sporangia were present in the hanging drops containing plants from *Chorda*, not a single zygote was present in these drops. These observations thus showed that the haploid plants on *Chordaria* were in reality sexual while the diploid plants on *Chorda* had for two seasons proved themselves to be asexual.

An experiment performed at Cuttyhunk indicates that the previous failures to obtain liberation of zoids from the plants growing on *Chordaria* may be owing to the fact that *Ectocarpus* was left attached to the host. If *Ectocarpus* is removed from *Chordaria*, placed in dishes and the water changed twice a day, it liberates gametes and remains healthy for several days; while plants which are attached to *Chordaria* and kept under similar conditions fail to liberate gametes after the second day. *Chordaria* when kept in an aquarium exudes a gelatinous substance which may have a pathological effect on *Ectocarpus* and thus prevent the liberation of zoids.

The early part of October, 1932, was spent at Penikese in order to study the following questions with respect to the sexual plants: (1) Are the plants monoecious or dioecious? (2) Do unilocular sporangia occur on haploid plants? (3) Do the sexual plants have certain plurilocular sporangia which produce asexual spores and not gametes? (4) Are either or both female and male gametes capable of parthenogenetic development?

To determine whether the sexual plants are monoecious or dioecious, single branches were cut off under a dissecting microscope, transferred to a drop of water in a watch glass, and reexamined to make certain that only one branch was included. After a single branch had been obtained, it was washed in filtered sea water and cut into two pieces. One of the pieces was then placed in a drop of filtered sea water on a cover glass and a hanging-drop culture designated *A* was made of it. In a similar way a second hanging-drop culture *B* was made of one of the two pieces of a branch from another plant. The remaining two pieces of the respective branches were combined in a culture *AB*. In this way three hanging-drop cultures, *A*, *B*, *AB*, were obtained from two branches of separate plants of *Ectocarpus*.

In order to guard against the possible presence of stray gametes in the hanging drops, the cultures were made in the late afternoon or evening after liberation of gametes had ceased for the day. Each slide was furthermore examined under a compound microscope to insure that no gametes were present.

Cultures made in this manner gave conclusive results, for it was observed that zygotes were formed each morning only in the hanging drops which contained pieces from two separate plants, and in no instance did zygotes occur in the drops containing a piece from but one plant. It was thus decisively established that the sexual plants are dioecious. By transferring the small pieces to fresh hanging drops once or twice a day, it was possible to obtain liberation of gametes from some branches for several days.

In certain of the suspended drops containing two pieces from separate plants of *Ectocarpus*, zygotes were not formed although gametes were present. The absence of zygotes in such drops was rarely due to the fact that only one of the two pieces had liberated gametes but usually because both pieces were of the same sex. If

zygotes were formed in a drop on one day but not on another, it was proof that only one of the associated pieces had liberated gametes in the latter case. Since the female gametes can be distinguished from the male gametes by the short period of motility of the former, it is a simple matter to determine whether the absence of zygotes in a hanging-drop culture containing two pieces from separate plants is due to the pieces being of the same sex. Furthermore, a microscopic examination of the gametes in the two drops which contain the other half of the respective branches will show that the gametes in both drops are of the same sex. The sex of the gametes in a drop containing pieces of two branches from separate plants can be determined also by experiment as follows: Gametes were present in the cultures *G*, *GH*, and *H*, but none of these contained zygotes. The gametes were all female, judging from the short period of motility. The following morning gametes were again liberated in the three respective cultures and the gametes from a branch *E*, which had previously shown itself to be male, were added by means of a small pipette to the cultures *G* and *H*. After a few minutes, conjugation commenced in these two drops while no fusions occurred in the drop *GH*. Thus it was conclusively proved that the gametes from the branches *G* and *H* were female.

From the writer's observations there is no indication of a "relative sexuality" such as HARTMANN (5) found at Naples, but the evidence at hand is not sufficient to preclude the possibility of its occurrence.

The assembling of large numbers of male gametes around a female as figured by BERTHOLD (2) was not observed. In no instance did more than about six male gametes gather around a female simultaneously, and in many cases the female seemed to attract only one male. Conjugation was not observed to occur while a female gamete was still swarming but always took place after the female had become attached at the edge of the drop toward the source of light.

The actual fusion of gametes is a rapid process which at most does not last longer than 20 seconds; and the successive stages of conjugation of any two gametes, as are figured by BERTHOLD (2), follow each other so rapidly that they cannot be observed in the living state. But it was found that the process of fusion is greatly retarded if male gametes are added to a culture of female gametes which has been

liberated for about one hour. After this lapse of time, a firmer membrane has been formed around the female gamete, and as a result the coalescence of conjugants is accomplished more slowly. Under these conditions a male gamete may attach itself to a female gamete, perform the usual vibratory movements, and then suddenly move toward the latter as in normal conjugation; but owing to the thickened membrane of the female a coalescence does not occur and the male gamete immediately settles down motionless beside the female. It is evident that the chances of fertilization are greatly reduced by the thickening of the membrane which renders the female gamete incapable of fertilization not long after liberation. It is not known how long after liberation a male gamete remains functional.

The male gamete usually unites with the broad posterior end of the female, as was observed by BERTHOLD (2) and KUCKUCK (12). This is undoubtedly due to the fact, as BERTHOLD and KUCKUCK state, that the attached anterior end of the female is not accessible to the male.

There is considerable variation in size among the gametes of each sex, some being nearly twice as large as others. Large and small gametes arise from different gametangia respectively. At first it was thought that these large zoids were zoospores which serve to propagate the haploid generation; but it was later observed that the larger as well as the smaller zoids from the sexual plants function as gametes. In consequence zygotes of various sizes are formed: large, small, and intermediate ones, depending upon the conjugation of two large gametes, of two small gametes, or of a large with a small gamete.

Unilocular sporangia were not found on the sexual plants, and the only means by which these plants can propagate themselves is by the parthenogenetic development of the gametes of either sex, as will be discussed later.

#### C. MISCELLANEOUS OBSERVATIONS ON ASEXUAL AND SEXUAL PLANTS

MORPHOLOGY.—Asexual plants are more robust than sexual plants (fig. 15) and frequently attain a length of more than 7 cm., while the largest sexual plants collected were but 4 cm. long. The

cells of asexual plants are considerably larger than those of sexual plants and measure on an average  $50 \times 70 \mu$  while the cells of sexual plants average only  $44.5 \times 45.5 \mu$ . The primary difference between the cells of asexual and sexual plants is thus in the length of the cells. The breadth of many cells of the sexual plants exceeds the length, a condition less common in the asexual plants. The plurilocular sporangia of asexual plants (fig. 3) are considerably larger and vary more in size and form than those of sexual plants (fig. 6). The familiar ropelike intertwining of the main axes is also more characteristic of the robust asexual plants.

**DISTRIBUTION.**—Sexual plants are of limited distribution and were found only at Penikese Island where they occur in large quantities and exclusively on *Chordaria*. Asexual plants are more widespread and were collected on *Spartina* and *Chorda* at Woods Hole, on *Chorda* and *Chordaria* at Cuttyhunk, and on *Chorda* at Penikese. It is a striking fact that the asexual and the sexual plants grow side by side at the latter locality but are each confined to its own host.

**DISCHARGE OF ZOIDS.**—The liberation of zoids, especially of the sexual plants, occurs primarily during the early morning and forenoon; but if the plants are kept in darkness overnight and placed in fresh sea water when exposed to daylight, they can often be induced to liberate zoids at other times of the day. As a rule, the opening of the plurilocular sporangium occurs at the apex. The opening of the unilocular sporangium is always at the apex.

It was not observed that the content of the unilocular sporangium is expelled in bulk as was found by KNIGHT (7). The zoids are contained in a gelatinous mass which oozes out of the sporangium, carrying the zoids with it. As the mass increases in size on the exterior of the sporangium, stages such as are shown in KNIGHT's figures 33-35 may be observed. In many instances the perforation is very small and each zoid becomes constricted to a dumb-bell shape as it passes through the pore. After liberation, the zoids remain in a spherical mass at the apex of the sporangium for a short time, then become free and swim off individually. The process of liberation frequently consumes more than two minutes and at times some of the escaped zoids have become motile while others are still within the sporangium. These observations on the liberation of zoids from

the unilocular sporangium were made on plants cultured in aquaria, and it is possible that this environment altered the normal mechanics of discharge.

As many as thirty-two zoids were counted from a unilocular sporangium of a plant from the sea while counts of four, eight, and sixteen zoids were made from the unilocular sporangia of plants grown in aquaria. The unfavorable environmental conditions of an aquarium have a dwarfing effect on the plants and their reproductive organs. It is doubtful whether unilocular sporangia from the sea contain fewer than thirty-two zoids and it is possible that as many as sixty-four may be formed in certain very large unilocular sporangia.

PERIOD OF MOTILITY OF ZOIDS.—Female gametes have the shortest swarming period of all the zoids, and frequently become stationary at the edge of a hanging drop five minutes after liberation; about 15 minutes is their maximum period of motility. Male gametes, on the contrary, have the longest swarming period of all the zoids and at times are still motile eight hours after liberation. Zoids from the plurilocular sporangia of asexual plants often remain motile for 3-5 hours while the zoids from the unilocular sporangia seldom remain motile for more than 30 minutes.

PHOTOTACTIC RESPONSE OF ZOIDS.—Although the great majority of all zoids are positively phototactic, a certain proportion react either negatively or as "neutrals." No adequate explanation can be given for this difference of response, but certain observations suggest that a disturbance of the zoids during their swarming period may stimulate them to become negatively phototactic. If positively phototactic zoids are taken from a vessel and placed in a hanging drop, a large proportion of them become negatively phototactic. (KYLIN (14) has also found this to be true.) If, however, hanging-drop cultures are made in the evening from pieces of *Ectocarpus* and these cultures placed in a dark room with light entering only through a small window, practically 100 per cent of the zoids liberated the following morning are positively phototactic.

SIZE OF ZOIDS.—A considerable difference in size exists among the three types of zoids. The zoospores from the unilocular sporangium are large and measure on an average  $9.1 \times 18 \mu$ ; the gametes are small



and measure on an average only  $4 \times 7.4 \mu$ ; and the zoospores from plurilocular sporangia of asexual plants are intermediate in size and measure on an average  $4.8 \times 9.5 \mu$ . Large zoids from unilocular sporangia and smaller zoids from diploid plurilocular sporangia are shown in figures 16-18. The difference in size between the gametes and the asexual zoids may be seen in figures 14, 16, 17, and 18.

SEASONAL CYCLE.—Although the writer's data regarding the seasonal cycle of *E. siliculosus* are not entirely complete, certain facts are worth recording. At Woods Hole, in contrast to the several localities where previous investigators have worked, unilocular sporangia are common and can be found throughout the summer months. Plants bearing unilocular sporangia were collected for the first time during the third week of June, 1931; but since these plants contained many mature and empty unilocular sporangia, there is reason to believe that these organs had begun to appear earlier in the year. Unilocular sporangia evidently disappear in autumn at Woods Hole and remain absent during the winter. Plants collected at Woods Hole on October 30, 1931, bore very few of these organs but many plurilocular sporangia. (*E. siliculosus* was rare at this time of the year.) The latest collection of the year was obtained from a small amount of material received from Woods Hole on December 21, 1931. These plants bore many plurilocular sporangia similar to those shown in figure 1 but had no unilocular sporangia. Although unilocular sporangia were abundant on *Ectocarpus* at Penikese during the early part of September, 1932, they had disappeared completely by the first week of October of that year. During the latter half of September, 1933, unilocular sporangia were found at Woods Hole on two occasions only; and they were entirely absent on plants received from Woods Hole on November 27, 1933.

The evidence from three seasons' collecting thus indicates that unilocular sporangia occur in the Woods Hole region primarily during the summer months while diploid plurilocular sporangia occurred at all seasons at which collections were made (June 5 to December 21). It is possible that the asexual plants occur throughout the year at Woods Hole and that their rareness during winter is owing to the transitory habit of the hosts.

The available data on the seasonal occurrence of sexual plants

are too fragmentary to justify any conclusions. Plants with gametangia were first collected in July, 1931, again in September of 1931 and of 1933; and they were still abundant during the first two weeks of October, 1932.

### Cytology

**FIXING.**—The following fixing agents gave good results: (1) CHAMBERLAIN'S (3) chromo-acetic-osmic acid solution which consists of 97 parts sea water, 1 part chromic acid, 1 part glacial acetic acid, and 1 part of 1 per cent osmic acid. The time of fixation required is four to five minutes. (2) Allen's modifications of Bouin's fluid (17): (a) B-15, which is the original Bouin's fluid made up with sea water to which is added 2 parts of urea and 1.5 parts of chromic acid; (b) B-3, which consists of picric acid, saturated solution in sea water, 75 parts; formalin 15 parts; glacial acetic acid 10 parts; and chromic acid 1 part. The time of fixation required is one to two hours. Mitoses are more abundant if the material to be fixed is first kept in darkness for two or three hours, as is suggested by DAMMANN (4).

**DEHYDRATING.**—Taylor's method (17) for dehydration was followed with minor modifications. The material was left one-half to one hour in each grade of the alcohol, salt water, and fresh water, indicated in table I, and was allowed to remain in 70 per cent alcohol until dehydrated for imbedding.

In making paraffin sections, the trouble caused by epiphytic diatoms may be partly overcome if the material to be sectioned is treated for 24 hours with a 5 per cent solution of hydrofluoric acid in 70 per cent alcohol, and subsequently washed in 70 per cent alcohol.

**SECTIONING AND STAINING.**—The usual methods of clearing, infiltration, and imbedding were followed. By placing the material selected for sectioning in a small cloth bag and transferring the bag from grade to grade of the alcohols and xylols, much time is saved. The material is taken out of the bag when it is in the final xylol. Rubber tubing rings placed on glass slides coated with glycerine, as described by BAUMGARTNER and WELCH (1), were used as imbedding troughs.

The sections were cut  $3\text{--}4\ \mu$  thick. Since a few diatoms always remain attached to the material, a great deal of time lost in sharpening the knife can be avoided by using Wade and Butcher safety blades and the microtome blade holder of the same make.

The material fixed in chromo-acetic-osmic acid was bleached in a 5 per cent aqueous solution of hydrogen peroxide for one hour.

Feulgen's reaction (LEE 15, pp. 306-307, 437-438; MARGOLENA 16; KRAUSE 8, p. 1792; WESTBROOK 28) and Haidenhain's iron-alum

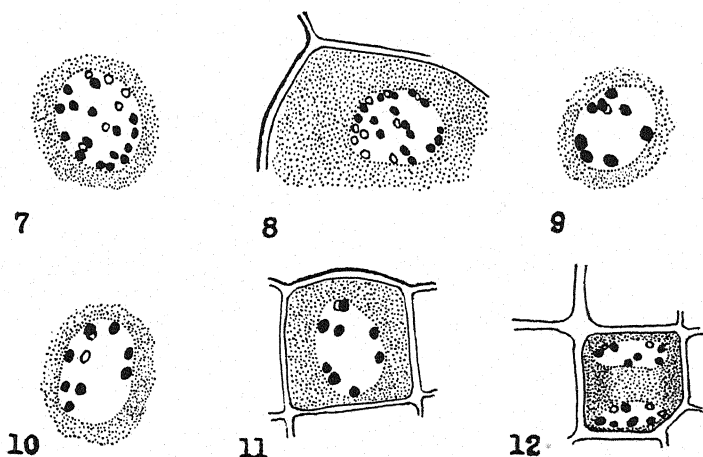
TABLE I

WATER, FRESH (PARTS)	WATER, SALT (PARTS)	ALCOHOL, 95 PER CENT (PARTS)
1	93	1
2	91	2
3	89	3
4	87	4
5	85	5
6	83	6
7	81	7
8	79	8
9	77	9
10	75	10
12½	70	12½
15	65	15
20	57½	17½
25	50	20
30	40	25
35	30	30
40	15	40
45	.....	50
35	.....	60
25	.....	70

haematoxylin were used as stains, but Feulgen's reaction gave the more satisfactory results. All the mitotic figures are drawn from preparations stained by this method. The chief advantage of Feulgen's reaction lies in the fact that, with correct treatment, it is practically specific for chromatin; and it leaves nucleoli and cytoplasmic inclusions unstained. The cytoplasm appears perfectly homogeneous and clear, especially if the material is fixed with B-15 or B-3. In this study the sections were treated with the 0.5 normal HCl for ten minutes and stained in the fuchsin sulphurous acid for four hours.

CHROMOSOMES.—The primary objective of this phase of the work was to determine whether the chromosomal cycle coincides with the

morphological phases of the life cycle. Although the results are incomplete in certain details, it has nevertheless been possible to establish that: (1) The asexual plants, with both plurilocular and unilocular sporangia, are diploid. No chromosome reduction occurs in the plurilocular sporangium and the nuclei in this organ contain about sixteen chromosomes (figs. 7, 8). In the unilocular sporangium on the contrary, one of the early nuclear divisions is undoubtedly a reduction division, for the nuclei which are formed later contain but



FIGS. 7-12.—Allen's fixative; Feulgen's reaction. Camera lucida drawings from paraffin sections: Fig. 7, polar view of early anaphase in plurilocular sporangium of asexual plant showing at upper level diploid number of chromosomes (16) and at a lower level 6 (unshaded) chromosomes of set for opposite pole. Fig. 8, anaphase from another plurilocular sporangium showing 16 chromosomes at upper level and 9 (unshaded) chromosomes of set for opposite pole. Fig. 9, polar view of metaphase in a unilocular sporangium showing haploid number of chromosomes (8 or 9), eight at upper level and one at lower level. Fig. 10, similar view, but from a different unilocular sporangium, showing haploid number of chromosomes (8) at upper level and two additional ones, probably of set for opposite pole, at lower level. Fig. 11, polar view of metaphase in a plurilocular sporangium of sexual plant showing haploid number of chromosomes (8 or 9); one is at a lower level than the other eight. Fig. 12, profile view of late anaphase or early telophase in a plurilocular sporangium of another sexual plant, showing haploid number of chromosomes (8) at each pole. All  $\times 4320$ .

eight or nine chromosomes (figs. 9, 10). (2) The sexual plants are haploid, the nuclei in the plurilocular sporangia of these plants containing eight or nine chromosomes (figs. 11, 12). Cytological obser-

vations thus show that there is an alternation of generations in the life cycle of *E. siliculosus* growing in the region of Woods Hole.

### Cultures

A. AQUARIUM CULTURES.—Two-liter battery jars were employed as aquaria, and these aerated by the aid of aspirators. The aqua-

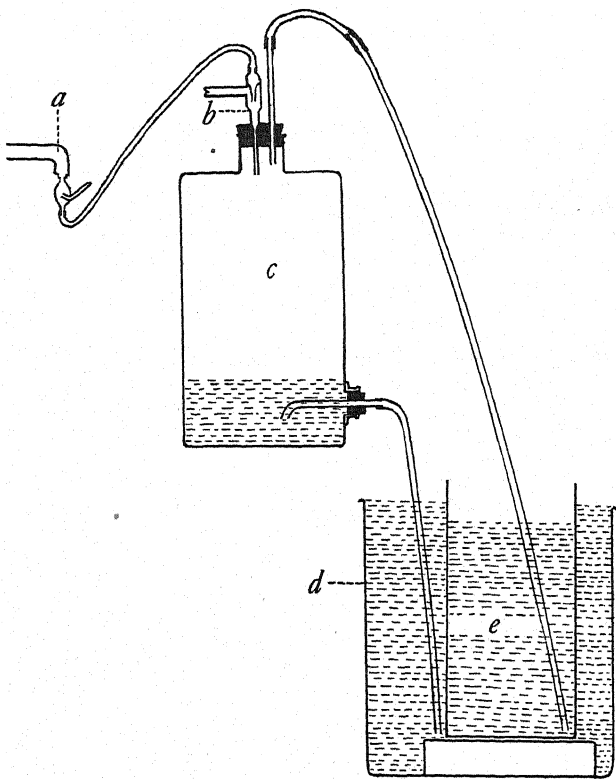


FIG. 13.—Diagram of apparatus for aerating an aquarium: *a*, faucet; *b*, filter pump; *c*, aspirator bottle; *d*, fresh water jar; *e*, salt water aquarium.

rium was kept cool by placing it within a larger jar and allowing the outflow of water from the aspirator bottle to run into the latter. Figure 13 illustrates the aerating system. The filter pump may be attached either to the faucet or, as in this case, to the aspirator bottle. Care has to be exercised not to place too much material in an

aquarium of this size. The best results are obtained by employing several aerating systems. As the sea water evaporates from the aquarium, distilled water should be added to prevent the sea water from becoming too saline.

By thus aerating and cooling the aquarium, it was possible to grow *Ectocarpus* for two months in the laboratory at The Johns Hopkins University; and the plants undoubtedly would have survived for a longer time if small crustaceans had not devoured them. Reproductive organs continue to be formed on plants growing in aquaria but they as well as the newly formed branches become dwarfed after a short time.

It was found that many unilocular sporangia are formed on asexual plants kept in aquaria. Plants collected at Woods Hole on October 30, 1931, bore but few unilocular sporangia and many plurilocular sporangia. After a week in the aquaria, these plants started to produce many unilocular sporangia while the plurilocular sporangia decreased in number, and after 14 days these plants bore decidedly more unilocular than plurilocular sporangia. Likewise, asexual plants which were collected at Penikese in October, 1932, and which contained no unilocular sporangia, started to produce these organs after they had been in an aquarium for two weeks. Sexual plants collected at the same time and kept in a similar aquarium failed to produce unilocular sporangia, a fact which gives additional evidence that unilocular sporangia are organs confined to the diploid asexual generation, as already discussed.

B. SILICA-GEL CULTURES.—Silica gel (26, 27) is a medium which deserves to be used more generally in the culturing of algae. The inorganic composition of this gel renders it superior to all other solid media in that it supplies no nourishment to the bacteria which are inevitably introduced into a medium with the spores of the alga to be cultured.

The following simple method for preparing the gel was shown to the writer by the Reverend A. KEEFE: Equal parts of sodium silicate, sp. gr. 1.1, and hydrochloric acid, sp. gr. 1.09, are mixed by stirring and the solution poured into petri dishes. The dishes are allowed to remain undisturbed for several hours, or preferably overnight, for the gel to set, then placed in a vessel and washed in running

fresh water for at least 24 hours to remove the chlorides. In order to have the gel saturated with sea water the dishes are left in a vessel containing filtered sea water for about 36 hours. The plates are then ready for inoculation or may be autoclaved before they are used.

It is necessary to add a liquid medium to silica gel plates since the gel dries within a comparatively short time. The liquid culture medium employed so successfully by SCHREIBER (25) for the culturing of *Laminaria* gametophytes was used. This liquid medium consists of:

NaNO <sub>3</sub> .....	0.1 gm.
Na <sub>2</sub> HPO <sub>4</sub> .....	0.02 gm.
Distilled H <sub>2</sub> O.....	50.0 cc.
Sea H <sub>2</sub> O.....	1000.0 cc.

Although plants have been raised from the spore to the formation of reproductive organs by this method, the writer has not yet obtained the entirely satisfactory results which may be expected from further experiment with this culture medium. A major source of trouble has been the rapid multiplication of diatoms which are introduced into the plates with the zoids.

C. HANGING-DROP CULTURES.<sup>3</sup>—The best cultural results were obtained from hanging-drop cultures. This method of culturing zoids is similar to the one already described for the observations on the behavior of zoids, with the exception that the parent plants are removed from the drops after liberation of the zoids has occurred and the slides are then placed in a damp chamber. The attached zoids adhere firmly to the glass, and it is possible to wash the covers by squirting sea water on them from a pipette, and thus rid them of most of the diatoms which are introduced with the parent plants.

It is necessary to dry the slides and covers frequently with filter paper, since the condensed vapor which accumulates on them tends to float up the cover slips and to flow into the hanging drops. Since the young plants adhere to the cover glass, it is possible to withdraw the drop of water with filter paper and replace it with fresh sea water from time to time without danger of losing the plantlets. In many cases the drop of sea water was replaced with SCHREIBER'S (25) culture fluid. Although diatoms multiplied rapidly in the silica gel

<sup>3</sup> A more satisfactory method for culturing algae is described by KYLIN (14) in a paper which appeared after this study had been completed.

cultures they never constituted a serious problem in the hanging-drop cultures. By the hanging-drop method plants were grown successfully from diploid zoospores, zygotes, parthenogenetic gametes, and haploid zoospores.

Diploid zoospores usually germinate within two to five hours after they have become attached. A series of early developmental stages of plants from these zoids is shown in figures 26-32. The plants shown in figure 19 have formed plurilocular sporangia. These plants previously also bore unilocular sporangia but unfortunately no photograph was taken of these earlier sporangia. In one case mature plurilocular sporangia were present on a 45-day old plant. In the majority of cultures, however, the young plants did not produce reproductive organs within the first two months. The  $F_1$  generation of plants from diploid zoospores is shown in figure 20. Plants grown in hanging-drop cultures are small but otherwise appear perfectly normal. Some of the plants were still in perfect condition when six months old.

The zygotes occasionally begin to germinate after nine hours but usually not before two or three days. The two eye-spots are often still visible after 48 hours. The sporelings from these structures develop very slowly as contrasted with those from diploid zoospores.

Only about 5 per cent of the unfused male and female gametes develop parthenogenetically. In a few cultures about 50 per cent of the unfertilized gametes germinated and it is possible that in the ocean considerably more than 5 per cent of the unfused gametes develop parthenogenetically. The parthenogenetic gametes are very slow in germinating. Occasionally the germ tube is discernible after 24 hours but more commonly it is 36 or 48 hours, or even more, before germination begins. The unfused gametes frequently enlarge to about twice their original size before they germinate.

The haploid zoospores from the unilocular sporangium usually begin to germinate within two to three hours after liberation. After eight hours the germ tube may be one-half the length of the zoid and after 24 hours the sporeling may have become a two-celled filament. Considerable variation in the rate of development exists, however, between individual sporelings. A series of developmental stages of plantlets from haploid zoospores is shown in figures 21-25.

After the cultures of plants from zygotes, parthenogenetic male



and female gametes, and haploid zoospores had been grown for two months without fruiting, they were accidentally allowed to dry up. Well developed vegetative plantlets were obtained from these cultures, however, and it can be concluded that zygotes, parthenogenetic gametes, and haploid zoospores, as well as the diploid zoospores, all play an active rôle in the life cycle of *E. siliculosus*.

From the observations on germination, it is evident that the initial growth of a sporeling always occurs from a germ tube arising at the narrow anterior end of a zoid, and after this tube has become several-celled, a second filament arises at the posterior end of the zoid (figs. 24, 25). A zoid always becomes attached in such a way that the narrow end is directed toward the incident light; and it seemed possible that the origin of the germ tube at this end of the zoid may be due to a photic stimulus. In order to test this, hanging-drop preparations were allowed to liberate zoids in a dark room lighted only from a very small window. After the zoids had settled at the edge of the drop toward the light, each with its narrow anterior end directed toward the window, the slides were turned through  $180^\circ$  and placed in a damp chamber in front of the window. Under these conditions the anterior end of the zoids, now directed away from the light, still continued to give rise to the initial germ tube. The position of the germ tube is thus independent of the direction of the incident light. Whether the place of origin of the germ tube is determined by a contact stimulus or by the polarity of the zoid is not known. In zygotes the germ tube arises from the anterior end of the female gamete. The further development of the small plants shows that they are positively phototropic, however, for the majority of filaments bend and grow toward the light. Figure 33 shows part of a hanging-drop culture treated in this way. The majority of filaments are growing toward the light and away from the edge of the drop. Figure 34, from the same culture but taken at the edge of the drop which is toward the light, shows that practically every filament is growing toward the light.

### Discussion

The results of this investigation show that there is an alternation of generations in the life cycle of *Ectocarpus siliculosus* in the region

of Woods Hole. The observations of three summers have shown, however, that there is a marked difference in the distribution of the two generations. Both generations grow at Penikese Island, 20 miles from Woods Hole, while only asexual plants occur at Woods Hole itself. Since large numbers of haploid zooids from unilocular sporangia are present at Woods Hole, the absence of sexual plants in this locality becomes a matter of considerable interest. The explanation of this condition was not forthcoming until the sexual plants were found at Penikese and it was observed that they are obligate epiphytes on *Chordaria flagelliformis*, an alga which does not occur at Woods Hole. The environment at Woods Hole is unfavorable for *Chordaria*, which usually grows in exposed localities like the one at Penikese.

The asexual plants of *Ectocarpus* occur at Penikese only on *Chorda filum* which grows mixed with *Chordaria*. This would seemingly indicate that the asexual plants are obligate epiphytes on *Chorda*. This is not the case, however, for these plants occur also on *Spartina* at Woods Hole and were found on one occasion on a small amount of *Chordaria* collected at Cuttyhunk Island.

It is not known whether the condition in the region of Woods Hole also applies to other localities, since the previous investigators, who found the sexual plants of *E. siliculosus*, do not specify the host. The dependence of the sexual generation on a specific host is not unique for *Ectocarpus*, however, but is also true for *Pylaiella littoralis*. KNIGHT (6) found that the diploid plants of *Pylaiella* occur on both *Fucus* and *Ascophyllum* while the haploid plants are confined to *Ascophyllum*, even when it grows mixed with *Fucus*. (KY-LIN (14) found that the haploid plants of *Pylaiella* do not grow directly on *Ascophyllum* but on *Sertularia pumila* which is attached to the latter.) It is possible that the dependence of the sexual generation on a certain host is a phenomenon of more common occurrence in algae than has been recognized.

The absence of the sexual generation of *Ectocarpus* in certain localities is due, undoubtedly, to an effect of the environment which inhibits the formation of unilocular sporangia. This is the case, apparently, at Kristineberg, Sweden, where there are only asexual plants which propagate themselves by means of zoospores from the

plurilocular sporangia (14). It is probable that a very slight difference in the environment may determine the absence of unilocular sporangia, for the writer found that asexual plants growing on *Spartina* at Grassy Island bore plurilocular sporangia only, while the plants which grew on *Chorda* in the same locality but in slightly deeper water bore both plurilocular and unilocular sporangia.

Since *Ectocarpus* has an alternation of generations in the region of Woods Hole, we would expect this to be true in other localities also, especially at Naples where sexual plants are abundant. Although there is as yet no record of an occurrence of asexual plants at Naples (except the plantlet shown in BERTHOLD'S (2) figure 8), it is evident that such plants exist there. Both BERTHOLD (2) and OLTMANNS (18) observed "neutral" spores from plurilocular sporangia, and OLTMANNS states that these are generally larger than the gametes, facts which indicate that these zoids were diploid zoospores. It is obvious that REINHARDT (20), SAUVAGEAU (22), and KUCKUCK (12) also had both generations of *E. siliculosus* at Sevastopol, Guéthary, and Helgoland respectively.

The plants investigated by KNIGHT (7) at Port Erin are similar to those occurring at Woods Hole proper, but the zoids from the unilocular sporangium behave entirely differently in the two localities, in that they function as zoospores at Woods Hole while they act as gametes at Port Erin. It is possible that the cases which KNIGHT designated as conjugations were abnormal fusions or sister zoids that had not become separated from each other in the sporangium. The following facts suggest that KNIGHT misinterpreted abnormal structures for conjugations: (1) fusions were not abundant; (2) conjugation occurred while the zoids were motile; (3) the process of fusion required about 20 minutes; (4) at times the zoids fused in groups. In contrast to this the writer found that many conjugations occur between the gametes from the plurilocular sporangia of haploid plants; conjugation always occurs between a motile male gamete and an attached female gamete; the process of fusion requires less than one-half minute; and the gametes never fuse in clumps.

KNIGHT believes that the unfused zoids from the unilocular sporangium die at Port Erin, but judging from the results obtained

at Woods Hole it may be concluded that the great majority of these zoids are capable of development into haploid plants. The absence of haploid plants at Port Erin may be due to the absence of the essential host as is the case at Woods Hole proper.

### Summary

1. An alternation of generations occurs in the life cycle of *Ectocarpus siliculosus* growing in the region of Woods Hole.
2. The diploid asexual plants bear unilocular and plurilocular sporangia on separate individuals or simultaneously on the same individual. In the plurilocular sporangium are formed diploid zoospores which germinate directly into other asexual plants.
3. A reduction division occurs in the unilocular sporangium and the haploid zoids formed in this organ germinate into haploid sexual plants.
4. The sexual plants are dioecious and produce physiologically anisogamous gametes in plurilocular sporangia. The zygotes develop into diploid asexual plants.
5. The sexual generation propagates itself by the parthenogenetic development of about 5 per cent of the unfused gametes of either sex.
6. Haploid plants are inferior to diploid plants in stature, size of cells, and size of plurilocular sporangia.
7. Plants were grown in suspended drops from spore to spore. Some cultures lived for more than six months.
8. Sexual plants were found only at Penikese Island where they occur on *Chordaria*. Asexual plants are widely distributed in the region of Woods Hole and occur on *Chorda*, *Chordaria*, and *Spartina*.
9. It is suggested that the sexual plants are obligate epiphytes on *Chordaria* and that their absence at Woods Hole proper is owing to the absence of this host in this locality.

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#### EXPLANATION OF PLATES VI, VII

All figures are unretouched photomicrographs of *E. siliculosus*.

##### PLATE VI

FIG. 14.—Zygotes (z) and unconjugated gametes (g). Note larger size and two eyespots of zygotes. Living hanging-drop culture.  $\times 504$ .

FIG. 15.—Asexual (2x) and sexual plants (x). Note difference in size. Formalin material.  $\times 7/10$  natural size.

FIG. 16.—Haploid and diploid zoospores, formed in unilocular and plurilocular sporangia respectively, of diploid asexual plants. Three large zoids are from unilocular sporangium. Living hanging-drop culture.  $\times 352$ .

FIG. 17.—Same but from a different culture. Ten large zoids from unilocular sporangium and two small zoids on left from plurilocular sporangium.  $\times 352$ .

FIG. 18.—Same but from a different culture, 5 hours after liberation of zoids. Two of the seven large haploid zoids from unilocular sporangium have already germinated.  $\times 332$ .

FIG. 19.—Plants from diploid zoospores bearing plurilocular sporangia. Living hanging-drop culture, 3  $\frac{1}{2}$  months old.  $\times 70$ .

FIG. 20.—Living  $F_1$  plantlets from zoospores of plurilocular sporangia formed on plants raised in hanging-drop culture from diploid zoospores.  $\times 184$ .

FIG. 21.—Living young plants from haploid zoospores. Hanging-drop culture, 3 days old.  $\times 344$ .

FIG. 22.—Living plantlets from haploid zoospores. Hanging-drop culture, 16 days old.  $\times 344$ .

FIG. 23.—Same, 21 days old.  $\times 352$ .

FIG. 24.—A 32-day old plantlet from a haploid zoospore. Living hanging-drop culture.  $\times 344$ .

FIG. 25.—Another plant from same culture and of same age.  $\times 344$ .

#### PLATE VII

FIG. 26.—Diploid zoospores, 10 hours after liberation: *c*, early stage in germination. Living hanging-drop culture.  $\times 384$ .

FIG. 27.—Same culture, 57 hours old.  $\times 384$ .

FIG. 28.—Same culture, 5 days old.  $\times 384$ .

FIG. 29.—Same culture, 13 days old.  $\times 374$ .

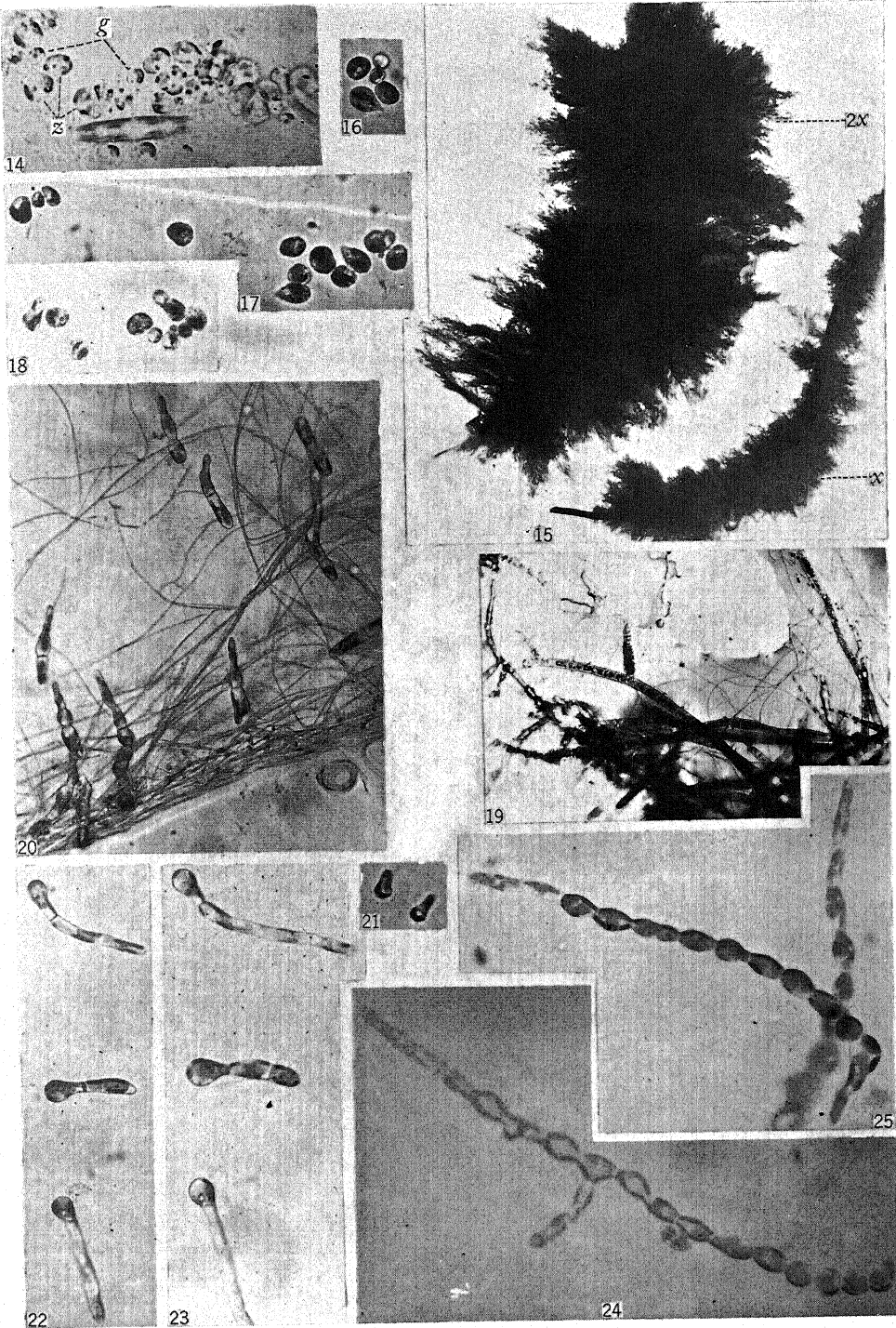
FIG. 30.—Living 14-day old plantlets from diploid zoospores. Hanging-drop culture.  $\times 80$ .

FIG. 31.—Living 22-day old plantlets from diploid zoospores. Hanging-drop culture.  $\times 378$ .

FIG. 32.—Living 30-day old plantlet from diploid zoospore. This sporeling developed unusually rapidly. Hanging-drop culture.  $\times 152$ .

FIG. 33.—Darker side of a 44-day old hanging-drop culture from diploid zoospores. This culture was kept in a dark room with light entering through a small window. After the zoids had become attached at edge of drop facing window, the slide was turned through  $180^\circ$ . The filaments are growing away from the edge of the drop and toward the source of light (indicated by arrow).  $\times 68$ .

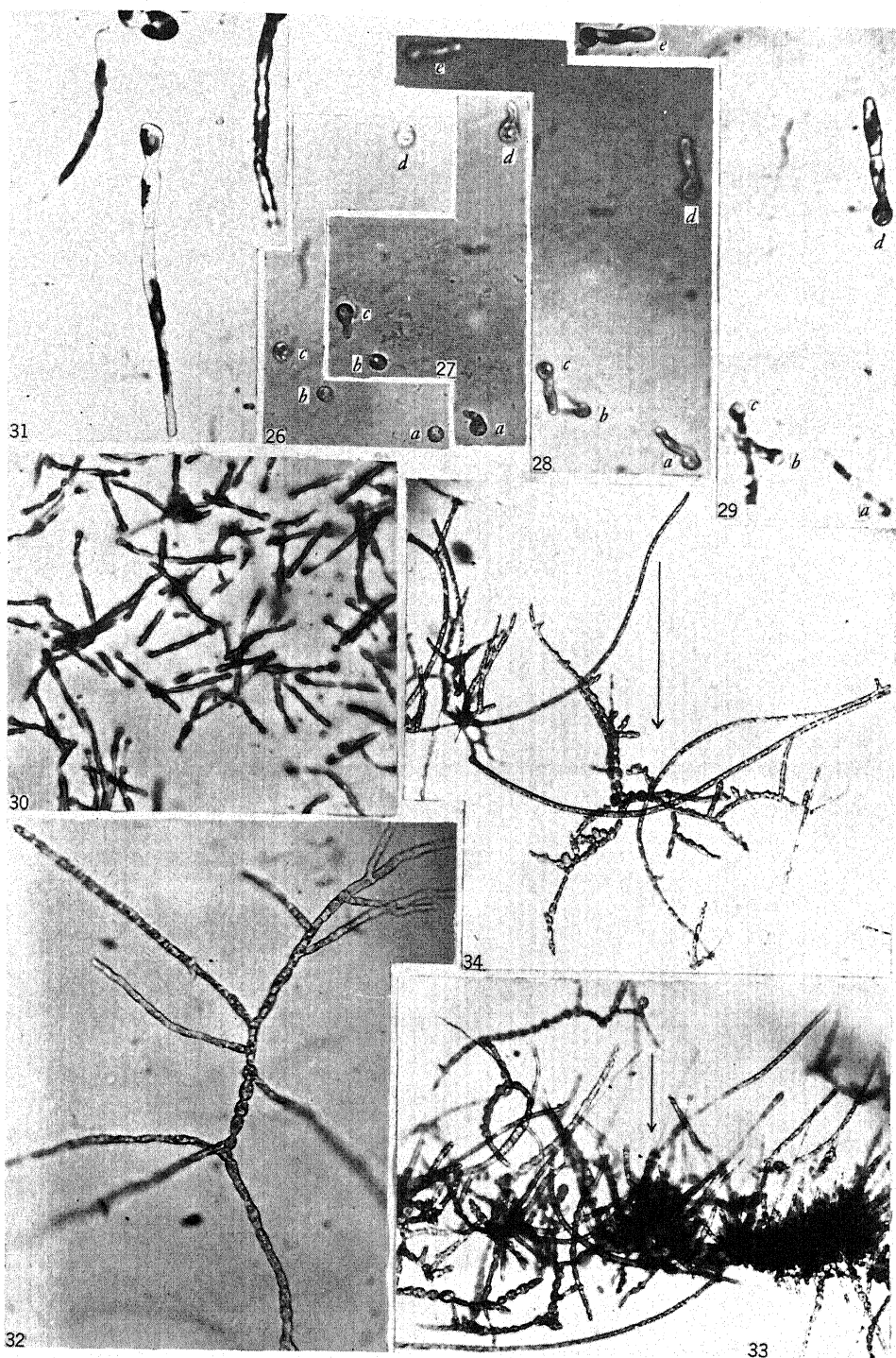
FIG. 34.—Illuminated side of same culture. The filaments are growing toward the edge of the drop (toward source of light).  $\times 68$ .



PAPENFUSS on ECTOCARPUS







PAPENFUSS on ECTOCARPUS



# IMPROVED METHODS FOR THE PURIFICATION OF THE COMMON CAROTENOIDS, AND THE QUANTITATIVE MEASUREMENT OF THEIR ABSORPTION SPECTRA

ELMER S. MILLER<sup>1</sup>

(WITH THREE FIGURES)

## Introduction

Experimental data obtained by STOKES (15) in 1864 suggested that plant chloroplasts contained two yellow pigments. Developing the method of partition between solvents, STOKES separated carotene and leaf xanthophyll, both of which he differentiated spectroscopically. He was not aware of the ease with which the carotenoids oxidize, and consequently his data lack much value because of the heat treatments and exposures to oxygen to which he subjected the compounds.

In 1883 BORDIN (1), by isolating two different types of crystals from plant extracts, demonstrated that the plant chloroplasts contain more than one yellow pigment. According to their solubilities, these crystals belonged to two different groups. To one group belonged the hydrocarbons (carotenes), which were very soluble in petroleum ether but only slightly soluble in ethanol; to the other group belonged the hydrocarbons with hydroxyl groups (xanthophylls), which were soluble in other alcohols but only slightly soluble in such solvents as petroleum ether.

After a study of the physical and chemical properties of plant pigments, TSWETT (17) introduced a new method for the separation of the component pigments. He filtered a moisture-free carbon disulphide (or petroleum ether) solution through a column of dry calcium carbonate, which was packed as tightly and evenly as possible. By this means a chromatogram was obtained and the pigments were differentiated into zones by virtue of this preferential adsorption of the calcium carbonate for the different pigments. By extracting these zones with suitable solvents, solutions of the different pig-

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ments were thus obtained. In addition to two chlorophylls, TSWETT succeeded in separating what he identified as several different xanthophylls. Although recent work indicates that some of TSWETT's xanthophylls were substances resulting from the oxidation of the carotenoids, his methods should be exhaustively reinvestigated. In 1909, ESCHER (2) developed a simple crystallization method for the isolation of carotene from carrot roots with excellent yields.

Recently KARRER (4, 5), KUHN (7, 8), and STRAIN (16) by chromatographic methods isolated alpha and beta carotene, but their publications do not include sufficient detail as to how the purifications were performed to make possible the confirmation of their results. Methods for the isolation of alpha and beta carotene and leaf xanthophyll are presented in this paper. Quantitative spectral analyses of binary mixtures of carotenoids have been made on pure substances thus obtained.

### Experimentation

In this paper the preferential adsorption method introduced by TSWETT is combined with parts of ESCHER's method. Neither of these workers conducted their purification in an inert atmosphere, a precaution which is employed in this study. The carotenoid solutions are never heated above 48° C. The tests employed for determining the purity of the carotenoids are the melting point, the optical rotation, and the absorption spectrum. Emphasis is placed on the last method because of the accuracy with which the specific absorption coefficients at any desired wave length may be measured by a recently developed spectro-photoelectric method (21).

PURIFICATION OF ALPHA AND BETA CAROTENES.—The methods presented here are based on 15 purifications (15 samples of each isomer) of the components, and are not only the shortest but the simplest methods found by the writer to give complete separation of the carotene isomers. The percentage composition of all solvents used is expressed on the basis of volume.

From 120 to 125 kg. of carrots are sliced and dried at 44° to 48° C. The oven employed for this purpose is equipped with a fan to insure the flowing of a continuous stream of warm air over the carrot pulp. Thirty to 36 hours is sufficient time for complete de-

hydration. The dried carrots are ground to a fine powder in a burr mill. The powder is placed in a continuous extractor and the pigments completely extracted by petroleum ether (b.p.  $60^{\circ}$ – $70^{\circ}$ ). Approximately 36 hours is required for extraction. Under diminished pressure at  $45^{\circ}$  C., the extract is concentrated to 2.5 liters. The solution after drying with sodium sulphate is filtered through a layer of calcium carbonate about 1.5 cm. thick. This carbonate filter is made in a Buchner funnel 19 cm. in diameter and 6 cm. in depth. The calcium carbonate layer is made by wetting the calcium carbonate with petroleum ether and applying suction to the Buchner funnel. As the petroleum ether is removed, the calcium carbonate is pressed down firmly until it forms a hard dry layer.

To the concentrated carotene solution, 25 gm. of potassium hydroxide is added. The saponification is carried out in a balloon flask for three hours at  $45^{\circ}$  C. The balloon flask is closed with a stopper fitted with a capillary outlet tube and a 3 mm. inlet tube which extends within 2 cm. of the bottom of the flask. During saponification, a stream of nitrogen is bubbled through the solution to insure a constant agitation and to exclude oxygen. After cooling the saponified solution, it is transferred to a 4 liter glass separatory funnel and washed six times with 500 cc. portions of distilled water. The major portion of the xanthophyll is removed from the carotene solution by washing the latter with seven 500 cc. portions of 89 per cent methyl alcohol. This fractionation is only approximate. After drying with 150 gm. of sodium sulphate, the carotene solution is filtered through another layer of calcium carbonate. The carotene solution is now a deep ruby red, and when held between the eye and a small electric filament lamp, the filament appears red and well defined. The solution is concentrated to 150 cc. under reduced pressure. The concentrated solution is now transferred to an Erlenmeyer flask, placed under nitrogen, and set in the refrigerator for 24 hours. The carotenes crystallize out with an appreciable amount of colorless impurities.

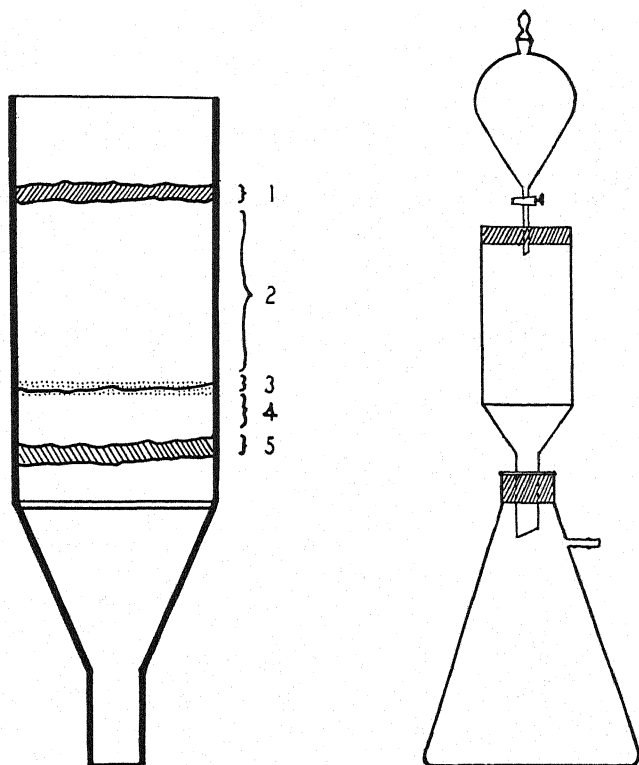
By filtration the carotene crystals are recovered on a filter paper in a Buchner funnel. After one more recrystallization from petroleum ether (b.p.  $60^{\circ}$ – $70^{\circ}$ ) the crystals melt at  $163^{\circ}$  C. The yield is approximately 3 gm. (end of ESCHER's method).

The major portion of the colorless impurities are removed by further recrystallization in an inert atmosphere. The crystals (0.60 gm. sample) are dissolved in boiling cyclohexane against which a stream of nitrogen is directed. As soon as a true solution is formed, the flask containing the carotene solution is quickly stoppered and immersed in a carbon dioxide-acetone bath. Within 10 minutes the solution is a solid. The solid solution is placed on a steam bath until approximately two-thirds of it has returned to the liquid phase. In this state the major portion of the impurities are insoluble in the cyclohexane and consequently form globules.

The solution is quickly decanted into a Buchner funnel containing a filter paper. The funnel is connected to a flask to which suction is applied. A stream of nitrogen is directed against the solution, which filters quickly. The globules pass readily through the filter paper which retains the crystalline carotenes. The crystals are washed with two 30 cc. portions of petroleum ether (b.p.  $30^{\circ}$ – $35^{\circ}$ ). The carotene crystals melt at  $176^{\circ}$ – $177^{\circ}$  C. Following the second recrystallization from cyclohexane as previously described, and a recrystallization from petroleum ether (b.p.  $60^{\circ}$ – $70^{\circ}$ ), the melting point is raised to  $181^{\circ}$  C<sub>cor.</sub>. The yield is 0.30 to 0.32 gm.

A carotene sample (0.31 gm.) is dissolved in the smallest possible amount of petroleum ether (b.p.  $60^{\circ}$ – $70^{\circ}$ ) and filtered through a 10 cm. layer of calcium hydroxide. This layer is made like the carbonate layer. For this purpose a glass funnel 14 cm. deep and 7 cm. in diameter (fig. 1) fitted with a sintered glass filter is used. The carotene solution penetrates the calcium hydroxide layer, forming a chromatogram (fig. 1). The adsorbed carotenes are washed with 150 cc. petroleum ether which aids in effecting a more complete separation. The lower zone may approach within 1 cm. of the sintered filter. If the carotene solution is too dilute, the thickness of the calcium hydroxide layer as described is not sufficient to adsorb the carotenes, and consequently some of the alpha carotene will occur in the last portion of the filtrate. It is impossible to have the carotene solution too concentrated for a satisfactory chromatographic separation of the isomers. If the calcium hydroxide layer is much thicker than 10–12 cm., the rate of filtration is too slow. Figure 2 illustrates the set-up employed for chromatographic analyses.

The top layer (fig. 1) is of a heterogeneous nature. The color of this zone varies from a deep bright red to a grayish red. Since this layer I contains the last traces of impurities and oxidized carotenes, it is removed with a spatula and discarded. Layer II contains beta carotene and is readily recognized by its deep orange color. It is quickly



FIGS. 1, 2.—Fig. 1 (left), carotene chromatogram; fig. 2 (right), adsorption apparatus.

removed and placed in an Erlenmeyer flask containing petroleum ether. If the carotene cannot be elutriated immediately, the adsorbed carotene should be kept in a stoppered flask under an inert gas. In order to obtain layers II and IV free from contamination, it is necessary to remove an intermediate layer III which includes the line of demarcation. Layer IV is removed with the same precautions



as layer II. Layer V contains an unidentified carotenoid which is discarded.

For the best results, the respective carotenes should be elutriated as quickly as possible from the calcium hydroxide. This is readily accomplished by stirring the solutions and filtering through the same funnel that contained the chromatogram. When removing the carotene from the calcium hydroxide in layer II, it is necessary to wash the calcium hydroxide with 500 cc. of petroleum ether containing 2 per cent methyl alcohol. About 125 cc. of this solution is sufficient to remove the alpha carotene from the adsorbent in layer IV. The respective solutions of alpha and beta carotene are concentrated under reduced pressure to one-eighth their volume, cooled, and set in the refrigerator. The carotenes usually crystallize overnight (in some instances it is necessary further to concentrate the solutions).

The carotene crystals are recovered in a Berlin crucible by filtration. KARRER (5) reports that a single filtration through a column of calcium hydroxide is sufficient for complete separation of the carotene isomers. The writer finds that a second filtration of solutions of alpha and beta carotene through a column of calcium hydroxide, as described, increases the purity of the components (fig. 3). After the second elutriation, the respective alpha and beta carotene solutions are again concentrated to one-eighth their volume, and the carotenes crystallize immediately. After a recrystallization from petroleum ether (b.p. 35°-60°) and drying for six hours in a vacuum desiccator, the carotenes give the following constants:

	MELTING POINT	$\alpha_{\text{Cd.}}^{20}$ (OPTICAL ROTATION)
Alpha carotene.....	187.5 $\pm$ 1° C.	390 $\pm$ 15
Beta carotene.....	187.5 $\pm$ 1° C.	0 $\pm$ 15

The yield is 19.5 mg. of alpha carotene and 113.0 mg. of beta carotene.

#### PURIFICATION OF LEAF XANTHOPHYLL

The method presented here was employed in the purification of twelve samples. Approximately 50 kg. of barley leaves (Wisconsin pedigree no. 38) are coarsely ground in a large food chopper and dried with the same precautions as carrot roots. The dried pulp is

again passed through the food chopper and then further pulverized by grinding in a burr mill. The total weight of the dried pulp is 4.0-4.4 kg.

The dried leaf powder is placed in a continuous extractor and the pigments completely extracted with acetone within 24 hours. After filtering the solution, it is concentrated to 1 liter under reduced pressure. Eighty grams of potassium hydroxide in 400 cc. methyl alcohol is added to the concentrated extract. The saponification is carried out as described in the purification of the carotenes.

After saponification, the solution is divided into two 700 cc. portions and each portion is transferred to a 4 liter glass separatory funnel containing 2 liters of distilled water. The carotenoids are removed from the saponified material by fractionation between carbon disulphide and the diluted acetone. It is necessary to pour the carbon disulphide through a small funnel in a manner which permits it to flow slowly down the side of the separatory funnel. After the separatory funnels are rotated gently, they are allowed to set for 30 minutes. The content of each separatory funnel is washed with three more 500 cc. portions of carbon disulphide. After the fourth portion is added, the separatory funnels are allowed to set for three hours before the carbon disulphide is drained off. The carbon disulphide portions are combined and washed with four 1 liter portions of distilled water. This removes the last traces of saponified material from the carotenoid solution. After the solution is dried with sodium sulphate, it is filtered through a 1.5 cm. layer of calcium carbonate. If the chlorophylls were not removed completely by saponification, they are removed in this filtration by preferential adsorption. The carotenoid solution is a deep clear red color.

After the solution is concentrated to 200 cc. under reduced pressure, 400 cc. of 89 per cent methyl alcohol is added and the solution is again concentrated to 200 cc. If all the carbon disulphide is not removed, another addition of 89 per cent methyl alcohol is made. The total volume of the solution is now increased to 2 liters by adding sufficient methyl alcohol to make the final concentration 89 per cent.

The carotenes are removed from the 89 per cent methyl alcohol solution by washing it with six 500 cc. portions of petroleum ether

(b.p.  $60^{\circ}$ – $70^{\circ}$ ). The carotenes from this stage and on are purified as previously described. The xanthophyll solution is concentrated to 75 cc. The precipitated xanthophyll is a yellow color owing to the dilution of the solvent with water. The xanthophyll crystals melt at  $168^{\circ}$  C. The yield of this crude xanthophyll is 1.4 gm. In all the following recrystallizations, the same precautions to exclude oxygen are employed as described in the methods for the purification of the carotenes.

A 0.25 gm. sample of the crude xanthophyll is dissolved in 50 cc. of boiling absolute ethyl alcohol to which 100 cc. of petroleum ether (b.p.  $30^{\circ}$ – $35^{\circ}$ ) is added. On cooling, the xanthophyll crystallizes immediately. After recovering the xanthophyll crystals by filtering this solution through a Berlin crucible, they are dissolved in 75 cc. of carbon disulphide. This solution is filtered through a 10 cm. layer of calcium carbonate which is made like the previously described 10 cm. calcium hydroxide layers. The adsorbed xanthophyll is washed with 25 cc. carbon disulphide. The resulting chromatogram consists of two or more zones. The top layer may vary from a white to a light red color depending on the kind and amount of impurities present. This layer is removed and discarded. The next zone, which comprises the major portion of the chromatogram, contains the adsorbed leaf xanthophyll.<sup>2</sup> Should other xanthophylls be present, the leaf xanthophyll will be in the lower zone (20). The color of the zone containing the leaf xanthophyll is a golden yellow. If carotenes are present the major portion will remain in the filtrate, and only a small amount will be adsorbed below the zone containing the leaf xanthophyll. After the leaf xanthophyll has been quickly elutriated with absolute ethyl alcohol, the xanthophyll solution is concentrated to 15 cc. under reduced pressure and set in the refrigerator. On cooling, crystallization of the xanthophyll occurs immediately. After recovering the xanthophyll crystals by filtration, they are washed with

<sup>2</sup> Since this paper was sent to press, it has been pointed out to the writer that the xanthophyll components observed by TSWETT should be exhaustively reinvestigated, especially in view of the fact that calcium carbonate is such a weak adsorbent. By the method presented here, the writer obtained the same absorption curve when leaf xanthophyll was isolated from either corn or barley leaves, which indicates that, if leaf xanthophyll consists of more than one component, the ratio between the components in this case remained fairly constant.

30 cc. of petroleum ether. After one hour's drying in a vacuum desiccator, the crystals melt at  $185^{\circ}\text{C}$ . The xanthophyll is dissolved in 22 cc. boiling absolute ethyl alcohol and cooled. To this solution is added an equal volume of petroleum ether, which causes the xanthophyll to crystallize. Following this crystallization, the xanthophyll is recrystallized from 17 and 15 cc. of methyl alcohol respectively. The final recrystallization is from 10 cc. of boiling carbon disulphide to which, after cooling, 30 cc. of petroleum ether (b.p.  $30^{\circ}\text{--}35^{\circ}$ ) is added. The dried xanthophyll crystals melt at  $190^{\circ}\text{C}$ . The yield is 95 mg.

The writer is indebted to several publications for the general method outlined here, the chief sources being the methods of WILLSTÄTTER and STOLL (19) and of SCHERTZ (13).

The lycopene employed in this study was furnished by MATLACK (9).

#### DISCUSSION OF CHROMATOGRAMS

The methods presented in this paper have shown that chromatographic analyses have extended applications in the purification of the carotenoids. The technique described, especially the packing of the adsorbent, was developed two years ago. The adsorbent then employed for the purification of the carotenes was "Fasertonerde" ( $\text{Al}_2\text{O}_3$ ). Recently, KARRER (5) has reported that calcium hydroxide is a satisfactory adsorbent. The difficulties encountered in chromatographic analyses are (a) the use of unsuitable adsorbents, and (b) improper packing of the adsorption column.

It was found that ordinary commercial slacked lime does not possess sufficient affinity for either of the carotenes to give a satisfactory chromatographic separation. This same difficulty is encountered with many of the commercial hydroxides. The purified calcium hydroxide distributed by Central Scientific Company was employed exclusively for the chromatographic analyses presented in this paper.

As previously described, the adsorption column must be packed uniformly and firmly. This is achieved by firmly packing 1 cm. layers of the wet adsorbent. The edges of each layer are packed the firmest, otherwise the lines of demarcation between the zones are very irregular. For a layer 10 cm. thick, the firmer the calcium hydroxide is packed the more complete will be the separation of the

carotene isomers. The same technique is used when Faserterde is employed as the adsorbent.

When purifying alpha carotene by the method (8) employing fuller's earth as the adsorbent, it is necessary to start with a 3 gm. sample of carrot root carotene. The low yield by this method is due to contamination by oxidized carotenes. These oxidation products are formed by progressive oxidation, while the carotenes are on the surface of the adsorbent. The presence of these impurities prevents a large portion of alpha carotene from crystallizing in later purifications.

In carrying out chromatographic analyses, certain precautions must be observed: (1) The original sample must be fairly free from impurities, the purification being carried out in an inert atmosphere to minimize the formation of oxidation products. (2) The adsorbent as well as the carotenoid solution must be free from moisture. (3) The adsorbent should be finely powdered, enabling close packing of the particles and consequently the formation of a firm layer of the adsorbent. (4) The solution in the funnel containing the chromatogram must be protected from exposure to oxygen (fig. 2). If the formation of oxidation products is not prevented or removed as formed, the resulting carotenoid preparation after elutriation will fail to crystallize. (5) It is necessary to wash the chromatogram to ensure complete separation of the components. As washing proceeds, the different zones move down but retain their relative position to one another.

#### DISCUSSION OF YIELDS

The yields of crude carotenes and xanthophyll by the methods presented here compare favorably with the highest reported in the literature. ESCHER found that carrot roots contained 0.0265 per cent as compared with 0.024 per cent by this method. The writer found that barley leaves contained 0.032 per cent (amount isolated) as compared with 0.012 and 0.04 per cent by the methods of WILLSTÄTTER and MEIG (18) and JØRGENSEN and STILES (3) respectively. In the latter method the source of the carotenes was nettle leaves. All these yields are on a dry weight basis.

The highest yield was that obtained after the final purification

of alpha carotene, namely, 0.0195 gm. of alpha carotene from 0.31 gm. carrot root carotene, a yield of 6.22 per cent. SMITH (14), starting with a 3.05 gm. sample of carrot root carotene, succeeded in isolating 0.028 gm., a yield of 0.92 per cent. Hence the method presented here increased the yield of alpha carotene approximately sevenfold. It is also significant that SMITH was unable to salvage any beta carotene from the same sample, whereas by the writer's method the yield of beta carotene from the same sample was 35.9 per cent.<sup>3</sup>

#### Absorption spectra of purified carotenoids

The absorption spectra of alpha and beta carotene, leaf xanthophyll, and lycopene were studied by the spectro-photoelectric method developed by ZSCHEILE, HOGNESS, and YOUNG (21); and by MILLER (12a).<sup>4</sup> For this study 20 per cent anhydrous ether and 80 per cent absolute ethyl alcohol were used as the solvent. The solutions were changed after every fifth reading to avoid photodecomposition of the carotenoids. The values of the absorption coefficients as defined by Beer's law are plotted as the ordinates of the curves. From Beer's law:

$$I_x = I_0 10^{-\alpha cx}$$

or

$$\alpha = \log_{10} \frac{I_0}{I_x} \frac{1}{cx}$$

$I_0$  = intensity of light transmitted by the solvent cell.

$I_x$  = intensity of light transmitted by the solution cell.

$x$  = thickness of the absorption cells (2.0 and 4.25 cm.).

$c$  = concentration of the carotenoids in gm. per liter.

The nature of the absorption curves determined the intervals at which the final measurements were made. From these measurements the values of  $\alpha$  were calculated and plotted as shown in figure 3.

<sup>3</sup> STRAIN has increased this yield. STRAIN H. H., Jour. Biol. Chem. 105:523-535. 1934.

<sup>4</sup> Curves presented in figure 3 were obtained as already described except that the electrometer was replaced by a modified DuBridge circuit.

For different wave lengths,  $\alpha$  was calculated from measurements made at different concentrations. When the values of  $\alpha$  were plotted against wave lengths, it was found that the values of  $\alpha$  for the different concentrations overlapped. This demonstrates that Beer's law is obeyed at the concentrations employed in this study, 0.0006 to 0.0012 gm. per liter. (The concentrations were limited by the thickness of the absorption cell employed.) The wave length readings are accurate to two Angstrom units. The experimentally determined values of  $\alpha$  at maxima on the curves have an error of 1.3 per cent or less and  $\pm 1.0$  per cent for the rest of the respective absorption curves.

The magnitudes of the absorption coefficients at different wave lengths were employed as the final criteria of purity of the carotenoids. On pure samples, it was found that the values of  $\alpha$  at certain wave lengths were approximately three times as sensitive and reliable as the criteria employing either melting points or optical rotation. The optical rotation method is reliable, but in this laboratory the spectro-photoelectric method was the most accurate. As the samples were purified, the resolution of the bands became more definite. The maxima of the bands increased and correspondingly the minima decreased, until the values of  $\alpha$  presented graphically in figure 3 were obtained.

McNICHOLAS (10) measured the absorption coefficients of carotene (mixture of alpha and beta carotene) and leaf xanthophyll with a Koenig-Martin spectrophotometer. Those measurements are open to criticism because recent investigations have shown that neither of the carotenoid preparations employed was pure. In 1933, SMITH (14) photographed the absorption spectra of alpha and beta carotene on Eastman no. 40 plates with a Bausch and Lomb no. 2700 spectrometer and a no. 2750 photometer. By these methods it is difficult to detect the small bands that can readily be located by the spectro-photoelectric method. Slit widths employed were 0.015 mm. or less. Spectral range isolated was 7.5 A. units or less.

More nearly accurate measurements of the absorption spectra of alpha and beta carotene were reported by KUHN (6). He employed a spectro-photoelectric method for measuring the absorption coefficients of alpha and beta carotene in hexane and carbon disulphide.

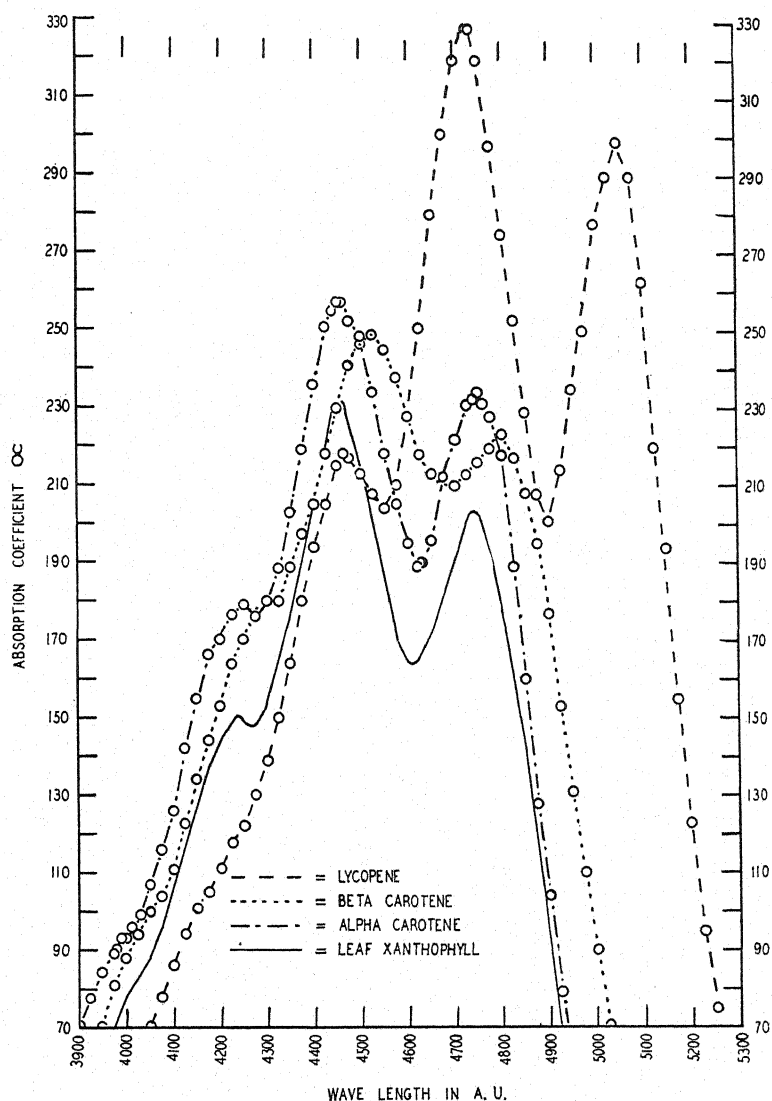


FIG. 3.—Absorption spectra of carotenoids in 20 per cent ether and 80 per cent absolute ethanol by volume.



The absorption spectrum of alpha carotene presented in figure 3 is similar to that obtained by KUHN. McNICHOLAS (10) and SMITH (14) failed to find the band at 4050 A. units for alpha carotene, and the band at 4085 A. units for beta carotene.

### Preparation of solvents

All solvents, as methyl alcohol, cyclohexane, chloroform, and petroleum ether fractions, were purified by careful distillations immediately before being used.

The acetone (c.p.) was redistilled. (b.p. 56.5° C.) The ammoniacal silver nitrate test for aldehydes was negative.

The ethyl alcohol (95 per cent) was allowed to stand over lime for four days. After removing the major portion of the lime by filtration, the ethyl alcohol was distilled.

The carbon disulphide had been used in previous extractions, but after distilling it boiled at 46.0°-46.4° C.

The diethyl ether (Baker's anhydrous) stood over sodium wire. It was distilled and stored in a brown bottle until used.

### Tests of purity

1. MELTING POINTS.—This method is valid only when a substance is free from contamination of impurities possessing the same approximate melting points.

2. TESTS FOR PRESENCE OF OTHER ISOMERS.—The contamination of one isomer with others can be ascertained by the proper chromatographic analysis. It can also be determined by tests 3 and 4.

3. OPTICAL ROTATION METHOD.—This method is very satisfactory when the proper sources of monochromatic light are used with a polarimeter capable of determining rotations as small as 0.01°. The apparatus employed in this study permitted measurements to be made with an accuracy of 4 per cent error; hence this test was not employed as the final test of purity.

4. ABSORPTION SPECTRUM.—A critical spectroscopic examination of the absorption spectrum measured with an error of 1.3 per cent or less at maxima readily detects traces of impurities and contamination by other isomers. This method is accurate and precise.

### Quantitative spectral analyses of binary mixtures of the purified carotenoids

A quantitative spectro-photoelectric method of analysis, in general, depends upon the precise determination of the fraction of light absorbed by a mixture of substances for at least as many different wave lengths as there are components for which analyses are to be made. The method may be exemplified by a brief consideration of the determination of the concentrations of alpha and beta carotenes in the same solution. In preparing for later analyses, it is first necessary to determine the absorption coefficients of pure components (alpha and beta carotene) in separate solutions (fig. 3) and over an extended wave length range. The purpose of this procedure is to permit the selection of the most suitable wave lengths of light to be employed in the analyses. These wave lengths are those for which there is an optimum difference between the absorption coefficients of the two components. Wave lengths 4862 and 4956 A. units were found to be satisfactory. Two or more wave lengths are employed for the purpose of check.

The average absorption coefficient of both carotenes in a mixture will be some value between the values of the coefficients of the pure components, and will be directly proportional to the relative amounts of the two components. The absorption coefficient of a mixture for a single wave length cannot be determined without a knowledge of the total amount of both carotenes, but if the absorption by the mixture is known for two wave lengths, then the absolute amounts of alpha and beta carotenes can be determined (two unknowns requiring two equations for solution). At 4500 A. units the absorption coefficients are equal for the carotenes. This wave length may be used to determine the total concentration, and one or more other wave lengths to determine the relative proportion of alpha and beta carotenes. The writer (11) has shown that for analytical purposes the percentage of composition of a component in a binary system can be calculated by the following equation:

$$a_T = (a_1 - a_2) \frac{c_1}{c_1 + c_2} + a_2 .$$

Tables I-III contain the results of analyses of several binary mixtures. The accuracy of the method may be seen by a comparison of the first and last rows. The last two columns (I and II) contain the determinations on different aliquots of the same unknown mixtures.

#### **Analyses of beta carotene and leaf xanthophyll in grass tissues**

A rapid and accurate quantitative method for extracting the carotenoids from green tissues has been described in detail by the writer (12). The weighed green samples are placed in a mortar (diameter 15 cm.) containing 25 cc. acetone and 25 gm. of quartz sand. The leaf tissue is macerated, and the acetone extract is decanted into a 250 cc. Erlenmeyer flask. The alternate maceration and extraction is repeated three times with 25 cc. portions of acetone and twice with 35 cc. portions of ether, the maceration and extraction requiring 12 to 15 minutes. Since all samples consist of succulent tissues, the sample is thoroughly disintegrated before the following extraction is made. The pulp and sand is collected in a washed cambray cloth bag which is placed in a Soxhlet extractor (size 18). About 100 cc. of ether is employed in transferring the pulp to the Soxhlet apparatus.

The extraction is completed by placing the Soxhlet extractor on a steam bath for 30 to 60 minutes. The quantity of carotenoids removed by the hot ether extraction is usually small. Analyses of several samples showed that this last extraction removed approximately 30 gamma from a 5.0 gm. sample. The chlorophylls and other esters are saponified by the addition of 20 cc. ethanol (95 per cent) saturated with potassium hydroxide to the combined ether and acetone extracts.

The extract is transferred to a 3 liter separatory flask containing 1.5 liter distilled water. The separatory funnel is whirled gently and allowed to set five minutes before the water is drained off into the second separatory funnel. If the saponification is not complete, an additional 10 cc. of ethanol saturated with potassium hydroxide is made. This second saponification may require 8 to 12 minutes. The content of the second separatory funnel is washed with 100 cc. ether. The ether washings are added to the first separatory funnel.

TABLE I

QUANTITATIVE SPECTRAL ANALYSES OF MIXTURES OF ALPHA AND BETA  
CAROTENE IN 20 PER CENT ETHER AND 80 PER CENT ETHANOL;  
COMPOSITION IN TERMS OF BETA CAROTENE

WAVE LENGTH (A.U.)	PERCENTAGE KNOWN COMPOSITION							EXAMPLES OF ANALYSES	
	5.0	10.0	25.0	50.0	75.0	90.0	95.0	I	II
4862.....	4.8	9.9	24.9	49.8	74.8	90.2	95.2	69.7	69.1
4956.....	5.4	10.1	25.1	50.0	74.8	90.3	94.8	67.5	68.2
Average percentage composition.....	5.1	10.0	25.0	49.9	74.8	90.2	95.0	68.6	68.7

TABLE II

QUANTITATIVE SPECTRAL ANALYSES OF MIXTURES OF BETA CAROTENE AND  
LYCOPENE IN 20 PER CENT ETHER AND 80 PER CENT ETHANOL;  
COMPOSITION IN TERMS OF LYCOPENE

WAVE LENGTH (A.U.)	PERCENTAGE KNOWN COMPOSITION							EXAMPLES OF ANALYSES	
	5.0	10.0	25.0	50.0	75.0	90.0	95.0	I	II
4956.....	5.2	9.8	24.9	49.7	74.6	89.7	94.6	30.5	29.6
5150.....	5.2	9.9	24.8	50.0	75.1	89.8	95.2	30.1	29.4
Average percentage composition.....	5.2	9.8	24.8	49.8	74.8	89.7	94.9	30.3	29.6

TABLE III

QUANTITATIVE SPECTRAL ANALYSES OF MIXTURES OF LYCOPENE AND LEAF  
XANTHOPHYLL IN 20 PER CENT ETHER AND 80 PER CENT  
ETHANOL; COMPOSITION IN TERMS OF LYCOPENE

WAVE LENGTH (A.U.)	PERCENTAGE KNOWN COMPOSITION							EXAMPLES OF ANALYSES	
	5.0	10.0	25.0	50.0	75.0	90.0	95.0	I	II
4722.....	5.4	10.2	25.6	50.6	75.7	90.0	95.4	34.5	34.1
5023.....	4.9	9.9	24.8	49.8	75.3	90.3	95.2	33.6	34.3
Average percentage composition.....	5.1	10.1	25.2	50.2	75.5	90.1	95.3	34.05	34.2

The ether solution in the first separatory funnel is washed four times with 500 cc. portions of distilled water. After each washing, the separatory funnel is stoppered and allowed to set two minutes, except the 5-8 minutes before the last washing is drained off.

TABLE IV  
QUANTITATIVE SPECTRAL ANALYSES OF THE COMMON CAROTENOIDS

PLANT	NUMBER OF SAMPLE	PERCENTAGE		
		BETA CAROTENE	LEAF XAN- THOPHYLL	ALPHA CAROTENE
Setaria stramineofructa.....	101	0.00753	0.03110	0.0000
	102	0.00740	0.03060	0.0000
Average.....		0.00746	0.03080	0.0000
Setaria italica.....	103	0.00840	0.02210	0.0000
	104	0.00820	0.02200	0.0000
Average.....		0.00830	0.02205	0.0000
Hordeum sativum (Wis. Ped. no. 38).....	105	0.00327	0.00840	0.0000
	106	0.00308	0.00840	0.0000
Average.....		0.00317	0.00840	0.0000
Dactylis glomerata.....	107	0.00408	0.01482	0.0000
	108	0.00443	0.01490	0.0000
Average.....		0.00425	0.01486	0.0000
Bromus inermis.....	109	0.00537	0.02360	0.0000
	110	0.00490	0.02410	0.0000
Average.....		0.00513	0.02380	0.0000
Zea mays.....	111	0.00454	0.02196	0.0000
(var. Country gentleman)	112	0.00470	0.02130	0.0000
Average.....		0.00462	0.02163	0.0000
Zea mays.....	113	0.00663	0.02185	0.0000
(var. Golden bantam)	114	0.00683	0.02250	0.0000
Average.....		0.00373	0.02217	0.0000
Festuca elatior.....	119	0.00604	0.02176	0.0000
	120	0.00625	0.02295	0.0000
Average.....		0.00615	0.02235	0.0000

The ether solution (150-200) is carefully transferred to a 500 cc. balloon flask, 25 cc. of ether being used for washing the separatory funnel. The ether solution in vacuo is evaporated to 50-60 cc. The carotenoid solution is transferred to a 100 cc. graduate and meas-

ured. The ether solution is poured into a 500 cc. volumetric flask. The balloon flask and graduate are washed with sufficient ether to make the total volume 100 cc. The solution is made up to volume by the addition of 400 cc. of absolute ethanol. It is usually necessary to make further dilutions before analyses are made. Quantitative spectral analyses of the carotenoids from eight grasses are summarized in table IV. Thus, by this spectro-photoelectric method it is possible to analyze plant extracts and to determine the total concentration of each component.

### Summary

1. Simple improved methods for the purification of three of the common carotenoids have been developed. The methods of TSWETT and ESCHER have been combined for the purification of the carotene isomers. The methods are described in detail.
2. A critical discussion of chromatographic analysis has been made. The necessary precautions are listed.
3. The quantitative absorption spectra of alpha and beta carotene, leaf xanthophyll, and lycopene were measured with an error of 2.0 per cent or less between 3950 and 5500 A. units. These spectra were measured by a spectro-photoelectric method which is accurate and precise.
4. Spectroscopic data show that certain tests of purity which have been considered accurate are only approximate. It is concluded that a critical examination of the absorption spectrum is the most reliable.
5. The absorption spectra of the carotenoids are employed as the basis for quantitative spectral analytical methods which have been applied to binary mixtures.
6. The preparative procedure includes and employs the precaution of excluding oxygen in the important steps of purification.

The writer wishes to express his appreciation to those who have given him the opportunity to make this investigation: to Prof. T. R. HOGNESS for his numerous suggestions regarding absorption spectra measurements and quantitative spectral analyses; to the Department of Botany, University of Chicago, for plants needed in the

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# TOXICITY OF PHENOLIC COMPOUNDS TO CERTAIN ONION BULB PARASITES<sup>1</sup>

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## I. Introduction

Resistance of colored varieties of onion to *Colletotrichum circinans* (Berk.) Vogl. is associated with the presence of water-soluble toxic substances in the dry outer scales of the bulbs (22, 23). Biochemical studies of extracts from colored scales have shown that the latter contain two phenolic substances, namely protocatechuic acid (3, 4 dihydroxy benzoic acid) (2, 13, 14) and catechol (1, 2 dihydroxy benzol) (15). These compounds, in concentrations of 1-800 and 1-1600 respectively, are strongly toxic to *C. circinans*, and are considered responsible, in part at least, for the resistance of colored bulbs.

The importance of phenolic compounds in plant tissue in warding off or restricting the attack of pathogenic organisms has been suggested by a number of workers. The work of COOK and TAUBENHAUS (5, 6), and COOK and WILSON (7) indicated that tannin compounds were toxic in certain concentrations to a great number of fungi. GRAVES (10) showed a distinct correlation of resistance to *Endothia parasitica* (Mur.) A. & A. with high tannin content of chestnut roots, while susceptibility of the bark of the stem was correlated with low tannin content. MARAÑON (16) found those strains of *Oenothera* which were resistant to *Erysiphe polygoni* DC. to be higher in tannin content than were susceptible strains.

Tannins and related compounds contain certain of the simpler phenolic compounds such as gallic acid, catechol, and protocatechuic acid. NEWTON and ANDERSON (17) offered the hypothesis that resistance in wheat to *Puccinia graminis* Pers. was due to phenolic compounds set free in the host cell by the action of fungal enzymes upon more complex substances. It was also suggested by them that

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susceptibility might be attributed to the presence of enzyme inactivators which prevented the fungus from setting free the phenolic compounds. Such a theory does not apply to the case of resistance in the onion. The phenolic compounds, catechol and protocatechuic acid, occur in the non-living tissue of the outer scales and diffuse into the soil moisture exterior to the colored bulb. If the fungus is present on the exterior its growth is inhibited and infection prevented. When this barrier of dead outer scales is removed, however, and the inoculum placed directly on the fleshy scales of colored bulbs, penetration and further invasion is exactly similar to that in bulbs of susceptible varieties. As pointed out in an earlier paper (22), the epidermal cells of the fleshy scales of colored varieties contain phenolic pigment compounds in the cell sap. The pigment is destroyed, however, as soon as penetration of the host cuticle is accomplished by the parasite and before the cell lumen containing the phenolic compounds is invaded by the fungus hyphae.

There is little experimental work to be found wherein the simpler phenolic compounds have been critically compared as to their effects upon fungous growth. NEWTON, LEHMANN, and CLARKE (18) reported studies with salicylic acid, catechol, and vanillin as they affected growth of *Helminthosporium sativum* P. K. & B. in Knop's nutrient solution. Salicylic acid was the most toxic and vanillin the least toxic. When added in very small amounts, however, all stimulated growth.

The present paper reports a study of the influence of twenty-one phenolic compounds upon four fungi. In addition to *C. circinans*, there were included two onion-bulb pathogens, *Botrytis allii* Munn and *Aspergillus niger* Van T. *Gibberella saubinetii* (Mont.) Sacc., a fungus pathogenic on wheat and corn but not known to attack the onion, was the fourth organism used. Colored onion varieties are not only highly resistant to *C. circinans* but they are also usually relatively free from infection by *Botrytis allii*. *Aspergillus niger*, however, attacks both colored and white bulbs (23).

## II. Methods

All the compounds studied were purified by appropriate methods until their physical constants corresponded to the accepted values

reported in the literature (1, 12, 20).<sup>2</sup> They were added in various amounts to modified Czapek's nutrient solution.<sup>3</sup> Fifty cc. aliquots of the medium were placed in 250 cc. flasks and sterilized in the autoclave. The inoculum for each flask consisted of 1 cc. of a suspension of the spores in sterile distilled water. The concentration of spores of a given organism was thus the same throughout any given dilution series. It was not the same for every experiment, however, and this accounts in part for the variation in the weights of the fungus between controls of different experiments with the same fungus. The cultures were incubated at room temperature, which varied from 22° to 24° C. in some of the series and from 26° to 28° C. in others. Each series was run in triplicate.

When the mycelial mats had reached the fruiting stage they were removed by filtering the cultures through weighed filter papers on a Büchner funnel. This was usually on the fourth day after inoculation with *Aspergillus niger* and on the sixth day with the other organisms. The paper and the fungus mat were dried in a vacuum oven at 80° C. under 12 mm. pressure and weighed.

With the data thus secured it was possible to compare the compounds with one another on the basis of the lowest concentration required to be incorporated in Czapek's solution for complete inhibition of germination and growth. This concentration will be referred to as the inhibitive concentration. It was also possible to compare the compounds as to the extent to which they retarded or stimulated growth of the fungi in Czapek's solution when present in the latter at various concentrations lower than that at which complete inhibition occurred.

### III. Results

The compounds studied will be considered in three groups. In the first group are the various phenols and substituted phenols; in the second are benzoic acid, various phenolic acids, and oxalic acid;<sup>4</sup> and

<sup>2</sup> Acknowledgment is made to Dr. SAM MORELL, Research Assistant in Biochemistry, for assistance in purification of the substances used and for the synthesis of the derivatives of protocatechuic acid.

<sup>3</sup> Magnesium sulphate, 0.5 gm.; monobasic potassium phosphate, 1.0 gm.; potassium chloride, 0.5 gm.; ferrous sulphate, 0.01 gm.; asparagin, 1.0 gm.; d-glucose, 30 gm.; water, 1000 cc.

<sup>4</sup> Oxalic acid was included because of its wide occurrence in plant tissues in the free state or as an oxalate, and also because of its toxic properties.

in the third group are a number of derivatives of protocatechuic acid. All four organisms were tested against most of the compounds. The data secured with *Colletotrichum circinans* are presented first, followed by the comparative results with the other organisms.

TABLE I  
GROWTH OF COLLETOTRICHUM CIRCINANS ON CZAPEK'S SOLUTION  
CONTAINING PHENOLS IN VARIOUS CONCENTRATIONS

PHENOL	DRY WEIGHT (MG.) OF FUNGUS AT VARIOUS CONCENTRATIONS											
	CON- TROL	1- 100	1- 200	1- 400	1- 800	1- 1600	1- 3200	1- 6400	1- 12,800	1- 25,600	1- 51,200	1- 102,400
$C_6H_5 \cdot OH$ Phenol	92 108 93	0 ..... .....	0 ..... .....	0 ..... .....	0 ..... .....	0 ..... .....	14 25 30	36 40 55	..... 60 60	..... 92 51	..... 80 77	..... ..... 73
$C_6H_4 \cdot (OH)_2$ 1:2 Catechol	89 176 135	..... ..... .....	0 ..... .....	0 ..... .....	0 ..... .....	0 ..... .....	10 23 10	25 62 29	..... 75 45	69 82 65	..... 95 80	..... 115 101
$C_6H_4 \cdot (OH)_2$ 1:3 Resorcinol	118 35	..... .....	0 .....	0 .....	44 14	75 17	93 24	103 29	122 30	119 35	140 .....	..... .....
$C_6H_4 \cdot (OH)_2$ 1:4 Hydroquinone	92 38	..... .....	0 .....	0 .....	0 .....	0 11	10 15	19 28	39 45	56 45	78 56	..... .....
$C_6H_3 \cdot (OH)_3$ 1:3:5 Phloroglucinol	36 35 93	6 ..... .....	13 10 36	15 17 66	21 18 88	25 29 115	23 36 111	26 38 115	..... ..... 142	..... ..... 151	..... ..... .....	..... ..... .....
$C_6H_3 \cdot (OH)_3$ 1:2:3 Pyrogallol	51 108	..... .....	..... .....	..... .....	0 .....	0 .....	0 .....	0 .....	0 .....	10 48	28 95	34 125
$C_6H_4 \cdot OH \cdot OCH_3$ 1:2 Guaiacol	135 176	..... .....	..... .....	..... .....	0 .....	0 .....	111 127	126 165	145 185	157 175	143 217	141 200
$C_6H_4 \cdot (OCH_3)_2$ 1:2 Veratrol	135 176	..... .....	..... .....	..... .....	0 .....	0 .....	136 155	138 165	133 155	144 183	135 161	141 171

TOXICITY OF PHENOLS TO COLLETOTRICHUM CIRCINANS.—The following phenols and substituted phenols were used: phenol; the three dihydroxy phenols, catechol, resorcinol, and hydroquinone; two trihydroxy phenols, phloroglucinol and pyrogallol; and guaiacol and veratrol, both of which are methyl derivatives of catechol. The results of growth studies in Czapek's solution are given in table I.

When these compounds are compared there appears to be no relation between molecular weight and toxicity. The position of the

hydroxyl groups in relation to one another, however, does have a distinct effect upon toxicity to *C. circinans*. The three dihydroxy isomers illustrate this fact clearly. Catechol, the ortho compound, was about equal to phenol. The inhibitive concentration was 1-1600. The weight of the fungus increased gradually with dilution of catechol but did not equal that of the control even at 1-102,400. Resorcinol, the meta compound, was much less toxic. The inhibitive concentration was 1-400 while normal growth occurred at 1-12,800 in one experiment and at 1-25,600 in the other. Hydroquinone, the para compound, was nearly as toxic as catechol and phenol.

A comparison of the two trihydroxy phenols shows a still wider range of toxicity between isomers. In phloroglucinol the hydroxyl groups are in the meta position to one another as in resorcinol, while in pyrogallol they are in the ortho position, as in catechol. Phloroglucinol was still less toxic than resorcinol. The inhibitive concentration was not determined, but growth occurred at 1-200 in three trials and at 1-100 in the one trial made at the latter concentration. Growth equal to the control occurred at 1-1600 in one trial and at 1-3200 in another. There was some stimulation at greater dilutions in one experiment. On the other hand, pyrogallol, with an inhibitive concentration of 1-12,800, was the most toxic of all phenols tested.

It is clear that, starting with phenol, the addition of one hydroxyl group in the meta position (resorcinol) reduces toxicity and changes the inhibitive concentration from 1-1600 to 1-400, while the addition of another hydroxyl group, in the meta position to the second (phloroglucinol), changes the inhibitive concentration to more than 1-100. On the contrary, when one hydroxyl group is added to phenol in the ortho position (catechol), toxicity is not changed materially, but when another hydroxyl is added in the ortho position to the second (pyrogallol), toxicity is increased abruptly and the inhibitive concentration is changed to 1-12,800. COOPER and MASON (8), in their studies with *Bacillus coli* and *B. fluorescens non-liquesfaciens*, found the toxicity of resorcinol and phloroglucinol less than that of phenol, and CAIUS, NAIDU, and SHAMSHER (4) reported the same for *Bacillus pestis*.

Guaiacol and veratrol are methyl derivatives of catechol. In the former, the methyl group is substituted for hydrogen in one of the

hydroxyl groups; in the latter, substitution occurs in both hydroxyl groups. In neither case was the inhibitive concentration changed from that of catechol. Retardation in growth, however, was more readily diminished with increase in dilution in the case of methyl derivatives than in that of catechol. At the 1-102,400 concentration of the latter, weight of the fungus was still less than that of the control. Guaiacol, however, did not retard growth at 1-12,800 and seemed to stimulate it at higher dilutions. Veratrol did not reduce growth at 1-3200 in one experiment and did so only slightly in another, but there was no stimulation at greater dilutions.

TOXICITY OF PHENOLIC ACIDS TO COLLETOTRICHUM CIRCINANS.—The following phenolic acids were studied: salicylic, meta-hydroxy benzoic, para-hydroxy benzoic, protocatechuic, gallic, and anesic. Benzoic and oxalic acids were added to the list for comparative purposes. The results of growth studies in Czapek's solution to which the acids were added in various concentrations are given in table II.

Benzoic acid was decidedly more toxic than any of its phenolic derivatives which were tested. The three isomeric monohydroxy benzoic acids differed from one another in toxicity and the variation resembled in a general way that which existed between the corresponding dihydroxy phenols. Thus salicylic acid, in which the hydroxyl and carboxyl groups are in the ortho position to one another, was the most toxic, with an inhibitive concentration of 1-6400. Para-hydroxy benzoic acid, in which they are in the para position to one another, was next in the descending order of toxicity, with an inhibitive concentration of 1-1600. Growth occurred in a 1-1600 concentration of the meta form, meta-hydroxy benzoic acid, and the inhibitive concentration, although not determined, was at 1-800 or greater. The monohydroxy acids therefore assume the same relative order of toxicity as the corresponding dihydroxy phenols: catechol, hydroquinone, and resorcinol. Salicylic acid reduced growth, as compared with the control, up to rather high dilutions (1-204,800); on the other hand, retardation of growth by the meta- and para-hydroxy acids was diminished more readily by dilution, for their effects were negligible at 1-6400 concentrations. This was also usually true for the meta- and para-hydroxy phenols, resorcinol and hydroquinone, as compared with the ortho form, catechol.

Only one dihydroxy benzoic acid (protocatechuic) and one trihydroxy acid (gallic) were available for study. It is thus not possible to compare either of these with its respective isomers. Protocatechuic acid was about equal in toxicity to meta-hydroxy benzoic acid, from which it differed only in the presence of a second hydroxyl group in

TABLE II  
GROWTH OF COLLETOTRICHUM CIRCINANS ON CZAPEK'S SOLUTION  
CONTAINING PHENOLIC ACIDS, BENZOIC ACID, AND OXALIC  
ACID IN VARIOUS CONCENTRATIONS

Acid	DRY WEIGHT (MG.) OF FUNGUS AT VARIOUS CONCENTRATIONS										
	CON- TROL	1- 400	1- 800	1- 1600	1- 3200	1- 6400	1- 12,800	1- 25,600	1- 51,200	1- 102,400	1- 204,800
$C_6H_5 \cdot COOH$ Benzoic	48 108	...	...	...	0 0	0 0	0 0	0 0	58 30	79 62	77 62
$C_6H_4 \cdot COOH \cdot OH$ 1:2 Salicylic	81 108	...	...	...	0 ...	0 0	53 20	56 32	67 38	...	63 90
$C_6H_4 \cdot COOH \cdot OH$ 1:3 Meta-hydroxy benzoic	42 107	...	...	...	13 80	39 136	30 146	32 149	46 191	...	...
$C_6H_4 \cdot COOH \cdot OH$ 1:4 Para-hydroxy benzoic	48 38	...	0 0	0 0	28 15	45 28	79 35	64 55	43 50	46 ...	...
$C_6H_3 \cdot COOH \cdot (OH)_2$ 1:3:4 Protocatechuic	36 81 31	0 0 ...	0 0 0	0 25 6	23 57 18	25 73 27	29 66 28	34 70 26	...	...	...
$C_6H_3 \cdot COOH \cdot (OH)_3$ 1:3:4:5 Gallic	78 35	0 0	15 11	25 16	20 25	35 20	42 35	54 48	...	...	...
$C_6H_4 \cdot COOH \cdot OCH_3$ 1:4 Anesic	108 42	...	...	...	...	...	...	8 ...	60 4	25 13	100 14
$(COOH)_2$ Oxalic	64 38	...	...	9 0	40 0	45 15	49 43	51 55	60 55	48 ...	52 ...

ortho position to the first hydroxyl. Gallic acid may be looked upon as protocatechuic acid in which a third hydroxyl group is added in the ortho position. This addition resulted in lower toxicity.

Thus we may point out the following series in which the addition of hydroxyl groups results in reduced toxicity as measured by the respective inhibitive concentrations: benzoic acid, 1-25,600; meta-hydroxy benzoic acid in which one hydroxyl group is added in the

1:3 position, 1-800 or higher; protocatechuic acid in which two hydroxyl groups are added in the 1:3:4 positions, 1-800; and gallic acid in which three hydroxyl groups are added in the 1:3:4:5 positions, 1-400.

Anesic acid, the methyl derivative of para-hydroxy benzoic acid, is distinctly more toxic than the latter. Oxalic acid is less toxic than benzoic and more toxic than the weakest phenolic acid tested (gallic).

TOXICITY OF PROTOCATECHUIC ACID DERIVATIVES TO *C. CIRCINANS*.—In the tests of phenols and phenolic acids just discussed certain methyl derivatives were included. The two derivatives of catechol (guaiacol and veratrol) prevented growth at the same concentration as catechol. At the higher dilutions, however, the toxicity of the methyl derivatives was more rapidly reduced and in the case of guaiacol there was some evidence of stimulation. On the other hand, anesic acid was much more toxic than para-hydroxy benzoic acid from which it is derived.

In view of our particular interest in protocatechuic acid, several of its derivatives were tested. These are listed and the data secured with each are given in table III. The acid prevented growth at 1-800. The methyl and ethyl esters were more toxic, both having inhibitive concentrations of 1-1600. The methyl derivatives (vanillic acid and veratric acid) were inhibitive also at 1-1600, but, like the methyl derivatives of catechol, the toxicity was reduced rapidly by dilution. Thus they were both decidedly stimulative at 1-12,800, while protocatechuic acid and the esters usually retarded growth perceptibly at 1-25,600. The diacetyl derivative was inhibitive at 1-3200 and retarded growth at dilutions as high as 1-51,200. Protocatechuic aldehyde inhibited the fungus at the same concentration as the acid but when diluted to 1-12,800 it stimulated growth. The methyl derivative of the aldehyde, vanillin, was slightly more toxic, but in one experiment stimulated growth at 1-6400 and lower concentrations.

SUMMARY OF RESULTS WITH *COLLETOTRICHUM CIRCINANS*.—It is shown in the foregoing experiments that phenolic compounds vary widely in their toxicity to *C. circinans*. Variation is as great between certain isomeric compounds as between more widely separated groups.



The position of the hydroxyl groups in relation to each other has an important bearing on the inhibitive concentration. Thus in the dihydroxy phenol and the monohydroxy phenolic acid series the

TABLE III

GROWTH OF COLLETOTRICHUM CIRCINANS ON CZAPEK'S SOLUTION CONTAINING PROTOCATECHUIC ACID AND CERTAIN OF ITS DERIVATIVES IN VARIOUS CONCENTRATIONS

COMPOUND	DRY WEIGHT (MG.) OF FUNGUS AT VARIOUS CONCENTRATIONS								
	CON- TROL	1- 800	1- 1600	1- 3200	1- 6400	1- 12,800	1- 25,600	1- 51,200	1- 204,800
$C_6H_3 \cdot COOH \cdot (OH)_2$ 1:3:4 Protocatechuic acid	36 81 31	0 0 0	0 25 6	23 57 18	25 73 27	29 66 28	34 70 26	.....	.....
$C_6H_3 \cdot COOCH_3 \cdot (OH)_2$ 1:3:4 Methyl ester of protocatechuic acid	36 31	0 0	0 0	12 13	30 28	25 31	27 30	.....	.....
$C_6H_3 \cdot COOC_2H_5 \cdot (OH)_2$ 1:3:4 Ethyl ester of protocatechuic acid	36 31	0 0	0 0	22 28	22 23	24 27	25 26	.....	.....
$C_6H_3 \cdot COOH \cdot (OC(=O)CH_3)_2$ 1:3:4 Diacetyl protocatechuic acid	36 31	0 .....	0 .....	0 0	19 15	24 20	26 20	26 24	31 31
$C_6H_3 \cdot COOH \cdot OH \cdot OCH_3$ 1:3:4 Vanillic acid	107 42	..... .....	0 .....	64 0	135 3	189 34	174 48	176 41	..... .....
$C_6H_3 \cdot COOH \cdot (OCH_3)_2$ 1:3:4 Veratric acid	176 135	0 0	0 0	17 19	26 33	196 180	200 193	180 178	175 133
$C_6H_3 \cdot CHO \cdot OCH_3 \cdot OH$ 1:3:4 Vanillin	42 107	..... .....	0 0	0 40	21 128	21 131	21 154	..... 204	..... .....
$C_6H_3 \cdot CHO \cdot (OH)_2$ 1:3:4 Protocatechuic aldehyde	93 42	0 0	3 0	55 6	83 40	126 55	135 55	..... .....	..... .....

ortho compounds exhibit the greatest toxicity while the meta compounds are the least toxic. In the trihydroxy phenols, phloroglucinol with a 1:3:5 arrangement of hydroxyl groups is very low in toxicity, while its isomer, pyrogallol, with a 1:2:3 arrangement is the most toxic of the phenols tested. Protocatechuic acid is about equal to

meta-hydroxy benzoic acid while the trihydroxy acid (gallic) is less toxic.

Guaiacol and veratrol, the methyl derivatives of catechol, have the same inhibitive concentration as the latter. Vanillic and veratric

TABLE IV  
GROWTH OF FOUR FUNGI ON CZAPEK'S SOLUTION CONTAINING  
PHENOLS IN VARIOUS CONCENTRATIONS

PHENOL	OR- GAN- ISM*	DRY WEIGHT (MG.) OF FUNGI AT VARIOUS CONCENTRATIONS										
		CON- TROL	1- 100	1- 200	1- 400	1- 800	1- 1600	1- 3200	1- 6400	1- 12,800	1- 25,600	1- 51,200
$C_6H_5 \cdot OH$ Phenol	C.c.	92	0	0	0	0	0	14	36	.....	.....	.....
	G.s.	123	0	0	0	0	0	32	71	.....	.....	.....
	B.a.	273	0	0	0	0	40	151	209	216	284	293
	A.n.	261	0	0	0	0	53	243	262	.....	.....	.....
$C_6H_4 \cdot (OH)_2$ 1:2 Catechol	C.c.	89	0	0	0	0	0	0	10	25	69	.....
	G.s.	63	0	0	0	0	0	16	30	36	45	.....
	B.a.	227	0	0	0	0	54	140	186	170	206	.....
	A.n.	162	0	0	47	104	155	142	145	159	145	.....
$C_6H_4 \cdot (OH)_2$ 1:3 Resorcinol	C.c.	118	.....	0	0	44	75	93	103	122	119	140
	G.s.	150	.....	0	49	77	104	117	125	157	149	163
	B.a.	274	.....	45	142	190	175	250	243	284	300	264
	A.n.	218	.....	0	164	154	169	155	219	215	211	220
$C_6H_4 \cdot (OH)_2$ 1:4 Hydroquinone	C.c.	92	.....	0	0	0	0	10	19	39	56	78
	G.s.	92	.....	0	0	13	37	31	45	42	46	44
	B.a.	120	.....	0	0	23	99	116	129	178	175	152
	A.n.	235	.....	161	164	172	190	215	215	213	178	203
$C_6H_3 \cdot (OH)_3$ 1:3:5 Phloroglucinol	C.c.	36	6	13	15	21	25	23	26	.....	.....	.....
	G.s.	169	115	133	127	143	134	148	139	.....	.....	.....
	B.a.	78	84	67	59	49	71	91	84	.....	.....	.....
	A.n.	266	224	238	230	221	220	219	245	.....	.....	.....
$C_6H_3 \cdot (OH)_3$ 1:2:3 Pyrogallol	C.c.	51	.....	.....	.....	0	0	0	0	0	10	28
	G.s.	107	.....	.....	.....	0	0	0	13	15	16	88
	B.a.	88	.....	.....	.....	0	0	0	4	6	8	26
	A.n.	91	31	44	49	84	104	121	89	90	88	.....

\* Abbreviations used in this table and table V are: C.c. = *Colletotrichum circinans*; G.s. = *Gibberella saubinetii*; B.a. = *Botrytis allii*; A.n. = *Aspergillus niger*.

acids, the methyl derivatives of protocatechuic acid, have a lower inhibitive concentration than the latter. When used in greater dilutions, however, the four methyl derivatives do not remain as toxic as the respective compounds from which they are derived. In fact they

TABLE V  
GROWTH OF FOUR FUNGI ON CZAPEK'S SOLUTION CONTAINING OXALIC, BENZOIC, AND CERTAIN  
PHENOLIC ACIDS IN VARIOUS CONCENTRATIONS

ACID	ORGAN- ISM*	DRY WEIGHT (MG.) OF FUNGI AT VARIOUS CONCENTRATIONS									
		CON- TROL	1-200	1-400	1-800	1-1600	1-3200	1-6400	1-12,800	1-25,600	1-51,200
$C_6H_5 \cdot COOH$ Benzoic	C.c.	48	.....	.....	.....	.....	.....	.....	.....	.....	.....
	G.s.	120	.....	.....	.....	.....	.....	.....	.....	.....	.....
	B.a.	125	.....	.....	.....	.....	.....	.....	.....	.....	.....
	A.n.	114	.....	.....	.....	.....	.....	.....	.....	.....	.....
$C_6H_4 \cdot COOH \cdot OH$ 1:2 Salicylic	C.c.	81	.....	.....	.....	.....	.....	.....	.....	.....	.....
	G.s.	105	.....	.....	.....	.....	.....	.....	.....	.....	.....
	B.a.	188	.....	.....	.....	.....	.....	.....	.....	.....	.....
	A.n.	205	.....	.....	.....	.....	.....	.....	.....	.....	.....
$C_6H_4 \cdot COOH \cdot OH$ 1:4 Para-hydroxy benzoic	C.c.	48	.....	.....	.....	.....	.....	.....	.....	.....	.....
	G.s.	110	.....	.....	.....	.....	.....	.....	.....	.....	.....
	B.a.	53	.....	.....	.....	.....	.....	.....	.....	.....	.....
	A.n.	107	.....	.....	.....	.....	.....	.....	.....	.....	.....
$C_6H_3 \cdot COOH(OH)_2$ 1:3:4 Protocatechuic	C.c.	81	.....	.....	.....	.....	.....	.....	.....	.....	.....
	G.s.	63	.....	.....	.....	.....	.....	.....	.....	.....	.....
	B.a.	176	.....	.....	.....	.....	.....	.....	.....	.....	.....
	A.n.	209	.....	.....	.....	.....	.....	.....	.....	.....	.....

\* For explanation of abbreviations used see footnote to table IV.

$C_6H_2 \cdot COOH \cdot (OH)_3$ 1:3:4:5 Gallic	C.c.	78	0	0	15	25	20	35	42	54	.....	.....	.....
	G.s.	76	0	23	25	69	92	77	88	78	.....	.....	.....
	B.a.	129	13	16	16	24	32	44	67	91	.....	.....	.....
	A.n.	184	204	209	240	243	253	278	313	317	.....	.....	.....
$C_6H_4 \cdot COOH \cdot OCH_3$ 1:4 Anesic	C.c.	42	.....	.....	.....	.....	.....	.....	.....	0	13	14	19
	G.s.	85	.....	.....	.....	.....	.....	.....	.....	0	73	78	75
	B.a.	188	.....	.....	.....	.....	.....	.....	.....	0	80	74	78
	A.n.	77	.....	.....	.....	.....	.....	.....	.....	53	66	43	44
$(COOH)_2$ Oxalic	C.c.	64	.....	.....	.....	9	40	45	49	51	48	52	.....
	G.s.	82	.....	.....	.....	20	27	38	43	47	69	89	.....
	B.a.	146	.....	.....	.....	113	125	158	144	161	192	195	.....
	A.n.	207	.....	.....	.....	179	209	203	212	229	242	253	.....

stimulate growth of the fungus in excess of the control at certain concentrations.

Other compounds in which there is a relatively narrow range between the inhibitive concentration and that at which growth is no longer retarded and sometimes stimulated are: meta-dihydroxy phenol (resorcinol), meta-hydroxy benzoic acid, protocatechuic aldehyde, and its methyl derivative vanillin.

TOXICITY OF PHENOLIC COMPOUNDS TO OTHER FUNGI.—As indicated earlier in the paper, the study of certain of the compounds was extended to *Gibberella saubinetii*, *Botrytis allii*, and *Aspergillus niger*. The results with phenols are given in table IV. In general the four fungi assume the following increasing order of tolerance to each compound: *C. circinans*, *G. saubinetii*, *B. allii*, and *A. niger*. One exception is resorcinol of which *B. allii* is more tolerant than is *A. niger*. The range of tolerance within this group of fungi was not the same for each compound. For instance, with pyrogallol the inhibitive concentration for *C. circinans* was 1-12,800, while for *A. niger* it was above 1-100. By contrast, the inhibitive concentration of phenol for *C. circinans* was 1-1600 and for *A. niger*, 1-800. In the case of resorcinol, where there was an indication of slight stimulation of *C. circinans* at certain dilutions, the same was true for *G. saubinetii* and *B. allii* but not for *A. niger*. *B. allii* was also stimulated at certain dilutions of phenol and hydroquinone while *A. niger* was stimulated by pyrogallol.

The results with certain of the acids are given in table V. The same relative order of toxicity of the acid compounds observed earlier with *C. circinans* held for the other three fungi. *C. circinans* again was invariably the least tolerant and *A. niger* was in all cases the most tolerant of the four organisms. Protocatechuic acid and gallic acid did not prevent nor retard growth of *A. niger* in concentrations as high as 1-200. In fact this organism was stimulated to greater growth than that of the control in all concentrations of gallic acid used. The tolerance of this organism to these phenolic compounds is not surprising in view of the fact that BUTKEWITSCH (3) found it could break down quinic acid to form protocatechuic acid and catechol.

#### IV. Discussion

A review of the literature reveals the fact that, in spite of the work of EHRLICH and others, our knowledge of the relations of the structure of phenolic bodies to their antiseptic and toxic properties is still very incomplete (9, 19). It has been shown by various investigators that the germicidal activity in homologous series of alcohols and phenols increases with molecular weight. JOHNSON and LANE (11) showed that the antiseptic strength of resorcinol increased as alkyl radicals of greater weight were introduced directly into the benzene nucleus. TILLEY and SCHAFER (21) found increasing germicidal value of normal alcohols with each additional methyl group in the chain. When isomeric alcohols were compared, however, a regular decrease in germicidal value from primary to secondary to tertiary normal alcohols was noted. The structure of the molecule and the molecular weight both influenced the degree of toxicity.

The concentrations necessary to prevent growth, as well as the growth rates at concentrations lower than that required for inhibition, were considered in the present study. In the compounds tested the molecular weight appeared to have little influence in determining relative toxicity. In the phenols the position of the hydroxyl groups in relation to one another affects toxicity. When the hydroxyls are arranged in meta position to one another there is a descending order of toxicity with increase in molecular weight, namely, phenol, resorcinol, phloroglucinol. When the hydroxyls are arranged in ortho position there is an ascending order of toxicity with increase in molecular weight, namely, phenol, catechol, pyrogallol. In the three isomeric monohydroxy benzoic acids the relation of the hydroxyl to the carboxyl group is the important structural feature of the molecules. Benzoic, meta-hydroxy, protocatechuic, and gallic acids make up a descending order of toxicity to *C. circinans* with increase in molecular weight.

The phenolic compounds vary with respect to the rate at which toxicity is reduced with dilution. Some retard growth over a wide range of dilution and do not stimulate growth in very dilute solutions. Others stimulate growth when diluted slightly beyond the inhibitive concentration. This is particularly true with some of the

more commonly occurring substances such as guaiacol, veratrol, vanillic acid, veratric acid, and vanillin.

The results of the present experiments show that the four fungi studied react quite differently from one another to the various compounds. If a longer list of fungi had been included a still greater range of tolerance might have been expected. Wide differences are found between onion parasites. For instance, protocatechuic acid inhibited *C. circinans* at 1-800 and retarded growth at 1-12,800 (table III), while *A. niger* was not retarded at 1-200 (table V). These data are in accord with the fact that colored onions containing this acid are very resistant to *C. circinans* and quite susceptible to *A. niger* (23).

It has frequently been inferred in phytopathological studies (5, 6, 7, 10, 16, 17, 18) that the mere presence in the plant of free phenolic substances, usually deduced from qualitative tests, or of compounds containing phenolic residues (the soluble pigments and tannins, for instance), might be regarded as means of protection against invading organisms. That these inferences are open to serious criticism is clear from the data presented here. It appears that the deciding factors are: (a) the specific phenolic substances present, (b) the concentrations in which they occur, and (c) their toxic or stimulative effect at such concentrations on the parasite in question. It is prudent to reserve judgment on the protective rôle of a phenolic substance (or substances) until it has been isolated from the host tissue in the pure state, whereupon its toxicity to the parasite in question, over a wide range of concentration, can be studied.

## V. Summary

1. The effect of twenty-one phenolic compounds upon the growth of four fungi (*Colletotrichum circinans*, *Gibberella saubinetii*, *Botrytis allii*, and *Aspergillus niger*) in Czapek's solution was studied.

2. In the phenol and phenolic acid series the position of the hydroxyl groups on the benzol nucleus is highly important in determining toxicity. Thus when the hydroxyls are arranged in ortho position to one another there is an ascending order of toxicity with increase in molecular weight: phenol, catechol, pyrogallol; while when they are in meta position to one another there is a descending order of toxicity with increase in molecular weight: phenol, resorcinol, phloroglucinol.

3. Some compounds tend to retard growth through a long series of dilutions beyond the inhibitive concentration, namely, phenol, catechol, salicylic acid. Others stimulate growth promptly in dilutions beyond the inhibitive concentration: guaiacol, meta-hydroxy benzoic acid, veratric acid, vanillic acid, and protocatechuic aldehyde.

4. Although with a few exceptions the organisms assume an increasing order of tolerance to each compound tested as follows: *C. circinans*, *G. saubinetii*, *B. allii*, *A. niger*, nevertheless there are often much wider differences among them in degree of tolerance to one compound than to another.

5. From these studies it is clear that many phenolic substances with a wide distribution in the plant kingdom exhibit little or no toxicity to certain parasitic organisms. Consequently the mere presence of phenolic substances in a host plant does not warrant the conclusion that they play a rôle in the resistance of that host to a given parasite or parasites. Toxic phenolic substances might be present in concentrations so low that their inhibitory effects are negligible, and they might also be present in concentrations that have a stimulative effect. When a phenolic substance with a specific toxicity toward a given organism is present in the host in an appropriate concentration, it may be regarded as part of the disease resisting mechanism of that host.

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# GENETICAL AND CYTOLOGICAL STUDY OF SPECIES HYBRIDS OF ASIATIC AND AMERICAN COTTONS

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(WITH TWENTY-TWO FIGURES)

## Introduction

The cultivated species of *Gossypium* have been divided into two groups, the Asiatic and the American (29, 16). Each of these groups includes several species; for example, of the common species *G. arboreum*, *G. nanking*, and *G. herbaceum* belong to the Asiatic group while *G. hirsutum*, *G. barbadense*, and *G. peruvianum* belong to the American group. Within each group the species cross readily but crosses between the different groups have been found difficult to make. As early as 1872, CLARK (cited by WATT 24) reported the latter as "not possible." Recently some successful crosses between Asiatic and American cotton species have been reported (25-27, 9), but the  $F_1$  plants showed complete sterility either when selfed or when back-crossed to the parental species. The reason for this is not yet known.

NIKOLJAVA (cited by ZAITZEV 25, 27), DENHAM (8), and BANERJI (2) reported different chromosome numbers in Asiatic and American species of *Gossypium*. The Asiatic group has 13 haploid or 26 somatic chromosomes, while the American group has 26 haploid or 52 somatic chromosomes. This throws new light on the problems of the difficulty of making successful crosses between Asiatic and American species and of the sterility of their hybrids, but the cytology of the sterile  $F_1$  hybrids was not reported until 1931.

The writer became interested in problems pertaining to the inter-specific hybrids between Asiatic and American cottons and started this work in June, 1931. The results obtained in the past two years are reported in this paper.

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## Literature review

1. INTERSPECIFIC CROSSES OF COTTON.—ZAITZEV (25-28) obtained two hybrids between *Gossypium herbaceum* and *G. hirsutum*. DESAI (9) obtained one hybrid plant from crosses between these two species. They both used *G. herbaceum* as the female parent. NAKATOMI (18) reported two successful hybrids between *G. hirsutum* and *G. herbaceum* and one between *G. barbadense* and *G. herbaceum*. HARLAND (15) reported two hybrids between *G. barbadense* and *G. arboreum* var. *sanguinea*. These two investigators also used Asiatic cotton as the female parent, but both met with no success. C. C. FENG (personal communication) obtained one hybrid between *G. hirsutum* (variety King) and *G. nanking* (variety Chinese White) while working at Georgia State College of Agriculture in 1920. ZAITZEV (28) described five natural hybrids between *G. herbaceum* and *G. hirsutum*. The present writer saw two natural hybrids between American and Asiatic cottons (most probably between *G. hirsutum* and *G. nanking*) in the summer of 1930 at the Experiment Station of National Central University, Nanking, China. In all these cases the hybrid plants showed considerable vigor and were self-sterile. When back-crossed to parental or other species, they were also sterile except in one case reported by HARLAND (15), who obtained eight plants as a result of back-crossing to *G. barbadense* with the pollen grains of the hybrids. SZYMANEK and GAVAUDAN (21) reported a fertile hybrid between *G. herbaceum* and *G. hirsutum*, and SZYMANEK (20) reported a fertile hybrid between *G. herbaceum* and *G. vitifolium*, but these results seem doubtful.

CYTOLOGICAL STUDIES ON COTTON.—CANNON (6) reported 28 as the haploid chromosome number of a hybrid between Sea Island cotton (*G. barbadense*) and Upland cotton (*G. hirsutum*). BALLS (1) reported 20 as the haploid chromosome number of an Egyptian variety (*G. peruvianum*). NIKOLJAVA (cited by ZAITZEV 25, 27) found that the somatic chromosome number is 26 in Asiatic cotton and 52 in American cotton. DENHAM (8) reported that Old World cottons have 13 haploid chromosomes, one being larger than the others; and that New World cottons have 26 haploid chromosomes with two larger than the others. BEAL (4) determined the haploid chromosome number of three American species as 26. BANERJI (2) confirmed the numbers given by DENHAM. BARANOV (3 = BARANOV,

MICHAILOVA, ELLENHORN, 1930; cited by EMME 11) found that the Old World cottons have 26 somatic chromosomes and the New World cottons have 52. The chromosome morphology was briefly described. In the  $F_1$  hybrid of *G. hirsutum*  $\times$  *G. herbaceum* BARANOV observed 13 bivalents and 13 univalents, the latter passing irregularly to the poles. ZHARBIN (cited by EMME) also counted 13 bivalents and 13 univalents in a *G. hirsutum*  $\times$  *G. herbaceum* hybrid. NAKATOMI (18) reported the haploid chromosome number of *G. herbaceum* to be 13 and that of *G. hirsutum* and *G. barbadense* to be 26. He described the chromosome behavior in microsporogenesis of  $F_1$  hybrids between Asiatic and American cottons. SZYMANEK and GAVAUDAN, and SZYMANEK reported variable chromosome numbers, for example, 10–13 haploid for Asiatic species and 20–26 diploid for American species. EICHHORN (10) published a brief note on somatic mitosis of *Gossypium* but no chromosome number was given. LONGLEY (17) reported 13 as the haploid chromosome number for Asiatic cottons and 26 for American cottons. He also made a fairly detailed description of the microsporogenesis of an Asiatic  $\times$  American  $F_1$  hybrid obtained by an accidental cross. SKOVSTED (19) reported 26 somatic chromosomes for Asiatic species. On the basis of chromosome configurations in a triploid Asiatic plant and other evidence, SKOVSTED suggested that "the Asiatic cottons ( $n=13$ ) must be polyploids." With a few exceptions (6, 1, 20, 21) the following points are agreed upon in these cytological studies: (1) the Asiatic species have 13 haploid chromosomes (26 somatic) and the American species have 26 haploid chromosomes (52 somatic); (2) the sterility of the Asiatic-American  $F_1$  hybrids finds a cause in the irregular chromosome behavior in the microsporocytes during the meiotic divisions.

#### Materials and methods

Typical Asiatic and American cotton species were obtained from agricultural institutions of China and the United States. Two species of Asiatic cotton, *G. arboreum* L. var. *neglecta* Watt. and *G. nanking* Meyen,<sup>2</sup> and two species of American cotton, *G. barbadense*<sup>3</sup>

<sup>2</sup> The scientific names for the Asiatic species given in this section follow the scheme of WATT (24). HARLAND (16) suggested that there is only one species in Asiatic cotton, *G. nanking* being reduced to a variety under *G. arboreum*.

<sup>3</sup> According to HARLAND (16).

and *G. hirsutum*, were used. In each species there were one to several varieties or strains. It was not possible to make several generations of selfing before the work was started, but the varieties used appeared to be uniform.

The crosses were made under greenhouse conditions. The methods of emasculation, pollination, tagging, and bagging were those employed by C. C. FENG (13). One precaution was added: before pollination the stigma was examined with a 10 $\times$  lens to see whether any stray pollen grains of the same flower were attached to it.

The cytological observations were made on slides prepared by the paraffin method. The somatic chromosomes were studied in root tips. Flower buds were fixed for studies of microsporogenesis, the bracts, perianth, and ovary being removed to facilitate killing. Fixation for both studies was mostly made in Nawaschin's solution. Bouin's solution was used once. Sections were cut 8-10  $\mu$  thick and stained with Haidenhain's iron-alum haematoxylin according to Sharp's schedule.

For studies of pollen tube growth, the flowers were artificially pollinated and kept in a controlled temperature room at 25° C. for 24 hours and then prepared according to the method given by BUCHHOLZ (5) for *Datura*. In cotton, however, there is great difficulty in removing the cortex of the style. Apparently there is no clear differentiation between cortex and conducting tissue like that in *Datura*.

### Asiatic-American hybrids

#### A. COMPATIBILITY

To test the compatibility between Asiatic and American species, 1708 interspecific crosses were made during the summers of 1931 and 1932. The results obtained are given in table I.

The hybrid bolls obtained were generally very small and contained only one to few seeds. Out of 29 bolls obtained in the last two years, 21 contained only one seed each, five contained two each, two contained three each, and one contained five seeds. When the seed was sown not every one germinated. Some were found to contain a very small embryo or none at all, even though they possessed well developed seed coats. Out of 22 ungerminated seeds examined in the

last two years, 13 were of this type. A few seeds had an embryo, but either the radicle failed to elongate or the seeds decayed. When the plants were grown, some were identical with the female parent, showed complete fertility, and gave no indication of hybrid vigor. These plants are most probably the result of accidental contamination by the pollen grains of the same flower or of the same variety. In all cases the pollen parent carried dominant factors which made

TABLE I  
RESULTS OF CROSSES BETWEEN ASIATIC AND AMERICAN  
SPECIES OF GOSSYPIMUM

PARENTS	CROSSES MADE	BOLLS OBTAINED	SEEDS OBTAINED	PLANTS GROWN	HYBRID PLANTS
ASIATIC×AMERICAN					
<i>G. arboreum</i> × <i>G. hirsutum</i> .....	54	0	0	0	0
<i>G. arboreum</i> × <i>G. barbadense</i> .....	1	0	0	0	0
<i>G. nanking</i> × <i>G. hirsutum</i> .....	836	2	2	2	1
<i>G. nanking</i> × <i>G. barbadense</i> .....	126	3	5	4	0
Total.....	1017	5	7	6	1
AMERICAN×ASIATIC					
<i>G. barbadense</i> × <i>G. nanking</i> .....	91	0	0	0	0
<i>G. barbadense</i> × <i>G. arboreum</i> .....	43	0	0	0	0
<i>G. hirsutum</i> × <i>G. nanking</i> .....	407	21	27	10	5
<i>G. hirsutum</i> × <i>G. arboreum</i> .....	150	3	8	4	0
Total.....	691	24	35	14	5
Grand total.....	1708	29	42	20	6

it possible to distinguish the true hybrids. In the last column of table I, the number of true hybrids is listed.

Table I shows that hybrid plants were obtained only from *G. hirsutum*×*G. nanking* and the reciprocal cross. It cannot be stated that these two species are more compatible than others, however, since more varieties and more plants were available for these two species and naturally more crosses were made between them. NAKATOMI (18) obtained one hybrid plant from *G. barbadense*×*G. herbaceum*, and HARLAND (15) obtained two hybrids from *G. barbadense*×*G. arboreum*. Sufficient data are not at hand to determine the difference in compatibility between different species.

Considering the compatibility of reciprocal crosses between Asi-

atic and American species, the cross of Asiatic ♀ × American ♂ does seem to be less compatible. Out of 1017 crosses with Asiatic cotton as the female parent, only one hybrid plant was obtained; while out of 691 crosses with American cotton as the female parent, five hybrid plants were obtained. This is a significant difference. NAKATOMI found more successful crosses with American cotton as the female parent. HARLAND (15) reported no success with Asiatic cotton as the female but obtained two hybrid plants with American cotton as female. This conforms to the general results of other interspecific crosses, in which greater success is obtained when the plant with the larger chromosome number is used as the female (22).

The writer was fortunately successful in obtaining hybrid plants from crosses in both directions; previous workers had reported success only with crosses in one direction or the other: Asiatic ♀ × American ♂ (ZAITZEV, DESAI); American ♀ × Asiatic ♂ (NAKATOMI, HARLAND).

Another difference between the reciprocal crosses is a difference in the retention of the young bolls on the plants after pollination. When American cotton was used as the female parent the unsuccessful ovaries dropped within two weeks after pollination. In the last two years only one or two small bolls (which gave no matured seed) out of 691 crosses were retained on the plant. In the reciprocal cross, with Asiatic cotton used as the female, 212 bolls out of 1017 crosses were retained on the plants and developed nearly to the normal size. These bolls did contain some weak fiber but no well developed seed; hence they could not be considered as successful crosses. This phenomenon has been mentioned by ZAITZEV (27, 28) and NAKATOMI (18). DESAI'S (9) use of retention of bolls as a criterion of success in an Asiatic-American cross is inaccurate.

In order to determine whether or not the low percentage of success in Asiatic-American species crosses is due to slowness of pollen tube growth, observations were made by methods used by BUCHHOLZ in *Datura*. Within 24 hours after pollination the pollen tubes of American cotton were seen traveling down to the base of the style of Asiatic cotton. When Asiatic pollen grains were placed on the stigma of American cotton, the pollen tube growth also has been followed to the base of the style. Sections of the ovary have

not been studied to trace the later development. The observations suggest that the low percentage of success in crosses between Asiatic and American species is due not to the slowness of foreign pollen tube growth, but to something else, probably an actual incompatibility of gametes.

#### B. HYBRID VIGOR OF $F_1$ PLANTS

Only three hybrid plants obtained from crosses made in 1931 have been studied. These were named hybrids A, B, and C. Hybrid A was the offspring of a cross with Asiatic cotton as the female parent,

TABLE II  
VEGETATIVE CHARACTERS OF  $F_1$  HYBRIDS COMPARED  
WITH THEIR PARENTS

PLANT	HEIGHT (CM.)	NUMBER OF NODES	LENGTH OF IN- TERNODES (CM.)	BASAL CIRCUM- FERENCE OF STEM (CM.)
Asiatic ♀ .....	93	20	4.65	2.3
American ♂ .....	48	22	2.18	2.6
Hybrid A .....	236	40	5.90	4.2
American ♀ .....	49	22	2.23	2.5
Asiatic ♂ .....	99	33	3.00	1.6
Hybrid B .....	203	39	5.21	2.9
American ♀ .....	49	24	2.04	2.5
Asiatic ♂ .....	99	25	3.96	2.5
Hybrid C .....	151	38	3.97	3.2

while B and C were from crosses where American species were used as the female parents. These were planted on February 15, 1932. Some measurements of vegetative characters made on November 20 of the same year are given in table II.

The three hybrid plants exceed their parents in each character listed. Hybrid A was more vigorous than the other two, suggesting that the reciprocal crosses may not give equal degrees of hybrid vigor. The hybrid obtained with Asiatic cotton as the female parent was more vigorous than those obtained with American cotton as the female parent. More such crosses are needed before it can be stated whether this is generally true or not.

Hybrid vigor of the  $F_1$  plants was reported by all the previous



workers who obtained Asiatic-American cotton hybrids. The increase in height is most conspicuous. In this case the increase in height is due to increased number of nodes as well as to an increase in the length of internodes, and not simply to an increase in the length of internodes as reported by WARE (23) in interspecific crosses with the American group.

### C. CHARACTERS IN $F_1$ HYBRIDS

The six hybrid plants obtained from the 1931 and 1932 crosses between *G. nanking* and *G. hirsutum* were studied. Two varieties of

TABLE III  
CHARACTERISTICS OF *G. NANKING* AND *G. HIRSUTUM*  
AND THEIR  $F_1$  HYBRIDS

CHARACTERISTICS	<i>G. NANKING</i>	<i>G. HIRSUTUM</i>	HYBRIDS
1. Hair coating . . . . .	Short and long hairs	Long hairs only	Long hairs only
2. Bract base . . . . .	United	Free	United
3. Bract teeth . . . . .	Short	Long	Long
4. Extra floral nectaries . . . . .	Absent	Present	Absent
5. Calyx teeth . . . . .	Short	Long	Long
6. Peduncle position . . . . .	Drooped	Erect	Intermediate
7. Leaf position at night . . . . .	Vertical	Horizontal	Vertical
8. Leaf lobe . . . . .	Narrower	Broader	Intermediate
9. Petal color . . . . .	Bright yellow	Cream	Dilute yellow
10. Petal spot . . . . .	Deep red	None	Faint red
11. Anther color . . . . .	Yellow	Cream	Yellow
12. Boll locks . . . . .	3-4	4-5	3-4

*G. hirsutum* (Acala and Trice) and four varieties of *G. nanking* (Million Dollar, Paoshan Whiteseed, Kwangsi Fuzzyseed, Chinting Large Yellowflower) entered into these crosses. The two varieties of *G. hirsutum* are identical in botanical characters and the four other varieties are identical also. Such characters provided in other varieties, as red leaf, small flower, white flower, red flower, were not utilized. The six hybrid plants showed the same characteristics and therefore may be studied collectively. The characters observed are summarized in table III.

Among these six hybrid plants one was obtained from a cross using Asiatic cotton as female while five were from crosses using American cotton as female. Their characters are the same. This shows that

reciprocal crosses give the same results even in crosses between species from different groups. Since these  $F_1$  plants are completely sterile, no further study of inheritance in  $F_2$  can be made.

### Sterility in $F_1$ hybrids

#### A. SELF-STERILITY

From June 1932 to January 1933, 227 flowers of the three hybrid plants have been selfed: 103 on hybrid A, 58 on hybrid B, and 66 on hybrid C. Not a single flower matured a boll, the young bolls falling off within two weeks after selfing. After January, 1933, some young bolls were retained on hybrid C, but these contained no developed ovules. Selfing hybrids D, E, and F also met with no success. This showed complete sterility on selfing, and confirmed the results of previous workers.

#### B. CROSS-STERILITY

Back-crosses to the parental species were made in both directions. One hundred and fifty-two flowers of the hybrid plants (73 on hybrid A, 49 on hybrid B, and 30 on hybrid C) were pollinated either by American or Asiatic species. One boll containing one matured seed resulted from pollinating hybrid C with *G. hirsutum*. This seed contained a good embryo but unfortunately did not germinate. Two other bolls resulting from pollinating hybrid C with Asiatic cotton were retained on the plant for 92 and 86 days respectively, but neither boll contained any well developed seed.

Two hundred and ninety-eight flowers of American and Asiatic species (212 on American and 86 on Asiatic) were pollinated with pollen from the hybrid plants. One boll containing six seeds was formed as a result of pollinating *G. hirsutum* (American) with hybrid C. These six seeds were planted in January, 1933, and five plants were grown. The plants were identical with *G. hirsutum* in every respect and were most probably the results of contamination by the pollen of the same species.

#### C. POLLEN ABORTION

The pollen grains of the hybrid plants are very irregular in size. The pollen grains of *Gossypium* are spherical in shape. Measure-

ments of diameters of the pollen grains of the parental species as well as of the hybrids were made in the summer of 1932. The results are given in table IV.

These figures (table IV) are based upon 100 measurements in each case and the unit is the micron. The pollen grains were killed by BOUIN's solution<sup>4</sup> to avoid bursting, and measurements were made under a microscope. The pollen grains of the parental species are very uniform in size, as shown by the low coefficient of variability.

TABLE IV  
POLLEN GRAIN SIZE AND VARIABILITY OF ASIATIC AND  
AMERICAN SPECIES AND THEIR HYBRIDS

PLANT	MEAN	RANGE	STANDARD DEVIATION	COEFFICIENT OF VARIABILITY
<i>G. hirsutum</i> .....	100.70 ± 0.28	88.0-110.0	4.09 ± 0.17	4.06 ± 0.19
<i>G. nanking</i> .....	87.79 ± 0.46	71.5- 99.0	6.88 ± 0.33	7.88 ± 0.38
Hybrid A.....	74.42 ± 1.00	44.0-132.0	14.84 ± 0.71	19.94 ± 0.99
Hybrid B.....	82.72 ± 1.59	33.0-143.0	23.54 ± 1.12	28.46 ± 1.46
Hybrid C.....	78.60 ± 1.22	33.0-126.5	18.06 ± 0.86	22.98 ± 1.15

The pollen grains of *G. nanking* are smaller than those of *G. hirsutum*. This may be due to the fact that the pollen grains of tetraploid plants are usually larger than those of diploid plants, although this rule does not apply universally. For example, the mean diameter of *G. barbadense*, another tetraploid species measured by the writer, is 113.41 ± 0.31  $\mu$ . The mean sizes of pollen grains of the three hybrid plants are even smaller than that of the Asiatic parent. The great irregularity is revealed by the range in size. The smallest size

<sup>4</sup> As this paper was being completed, attention was called to a paper by FERGUSON and COOLIDGE (14) on methods of studying the pollen grains of *Petunia*. They denounced the value of an aqueous medium in pollen grain studies because it changes the shape and size of the pollen grains. Since the pollen grain of cotton is spherical there is no distortion in shape as in the ellipsoidal *Petunia* pollen grain. For the size of the grain the writer found a 5% decrease in the diameter of the pollen grains of *G. hirsutum* and *G. nanking* when they were mounted in Bouin's solution as compared with those mounted in air. The coefficient of variability is nearly the same. This indicates that very little error is introduced when determining the size of cotton pollen mounted in Bouin's solution. Since material is not available, the variation of pollen grain size in the hybrids has not been checked.

attained was only about one-third of the mean diameter, and the largest ones were even larger than those of the American parent. They showed a high standard deviation and coefficient of variability as compared with those for the parental species.

When pollen grains of the parental species were placed in water on a slide they burst vigorously and discharged their contents. When pollen grains of the hybrid plants were mounted in water very few of them burst, and even then they discharged only a small amount of contents. Such a phenomenon has been reported by ZAITZEV (28). The irregularity in pollen grain size has been observed by ZAITZEV (28), NAKATOMI (18), and LONGLEY (17).

One characteristic of the hybrids not mentioned by previous workers is non-dehiscence of the anthers. The anthers of the normal cotton plants burst in the morning as soon as the flowers opened. The anthers of the sterile  $F_1$  hybrids generally dehisced later than those of the normal plants. Some anthers were shrunken in appearance and failed to dehisce. In winter when the plants were kept in a cool greenhouse ( $55^{\circ}$ – $65^{\circ}$  F.) there was a high percentage of flowers showing non-dehiscence of anthers.

#### D. OVULE ABORTION

The normal ovules are white and fleshy. A few days after pollination the beginning of the development of fibers on the seed coats is apparent. The ovules of the sterile  $F_1$  hybrids were shrunken, dry, and black; moreover, no fibers developed on the seed coats even though the bolls had been retained on the plant as long as three months. The ovules apparently do not develop at all. Comparing the retained bolls on the hybrid plants (*cf.* under A and B of this section) and those on the Asiatic species (*cf.* under A of section IV), the development of fibers and seed coats of the unfertilized Asiatic ovules was much more advanced than that in the hybrid plants. This indicates inability of the sterile ovules to develop.

Non-functional pollen grains and ovules were reported by most of the workers dealing with Asiatic-American hybrids. From DESAI's (9) statement that "The anthers appeared quite healthy and both the pollen grains and ovules did not show any obvious defects," it is doubtful whether he was dealing with a hybrid.

### Chromosome numbers of parental species

The somatic chromosomes of *G. nanking* and *G. hirsutum* were studied in root tips. *G. nanking* possesses 26 somatic chromosomes (fig. 3) and *G. hirsutum* 52 (fig. 4). This confirms the results of the Russian workers (NIKOLJAVA, cited by ZAITZEV 25, 27; BARANOV 3). The chromosomes of *G. nanking* are easily counted because the number is not large and the individual chromosomes are well separated. The chromosomes in *G. hirsutum* are rather difficult to count because of the large number and the fact that the nucleus is only a little larger than that of *G. nanking*, which makes the chromosomes much more crowded. The diameter of a nuclear plate of *G. nanking* is about 10  $\mu$  while that of *G. hirsutum* is about 12  $\mu$ . DENHAM (7) gave the somatic chromosome number of three varieties of American cotton as "circa 52" because he met with the same difficulty. SZYMANEK and GAVAUDAN's (21) report of 20-26 as the diploid chromosome number for *G. hirsutum* is probably incorrect. The writer never found such a small number in the root tips of *G. hirsutum*.

There are striking differences in size and shape between the chromosomes of the complement in each species. The longest chromosomes are three or four times as long as the shortest ones. Chromosomes with nearly equal arms and with unequal arms were observed. Some chromosomes appeared rod-shaped, probably owing to the fact that one of the two arms is very short. At the present time knowledge of chromosome morphology is not sufficient to enable one to distinguish the chromosome complements of any species of *Gossypium*.

The chromosome number of *G. nanking* was also determined in microsporocytes in several varieties. Figures 1 and 2 give two examples. Figure 1 was taken from a metaphase of the first division and figure 2 was from a metaphase of the second division. The haploid number is 13; this confirms the results of the previous workers. The 13 chromosomes stand out clearly and there can be no doubt as to the exact number. SZYMANEK and GAVAUDAN's count of 10-13 for *G. herbaceum* is doubtful.

The different sizes of chromosomes at the metaphases of the meiotic divisions are not very evident, as shown in figures 1 and 2.

This agrees with the observations of BANERJI (2). Although DENHAM (8) claimed that one chromosome is larger than the others, his figures (8, p. 435) are not convincing.

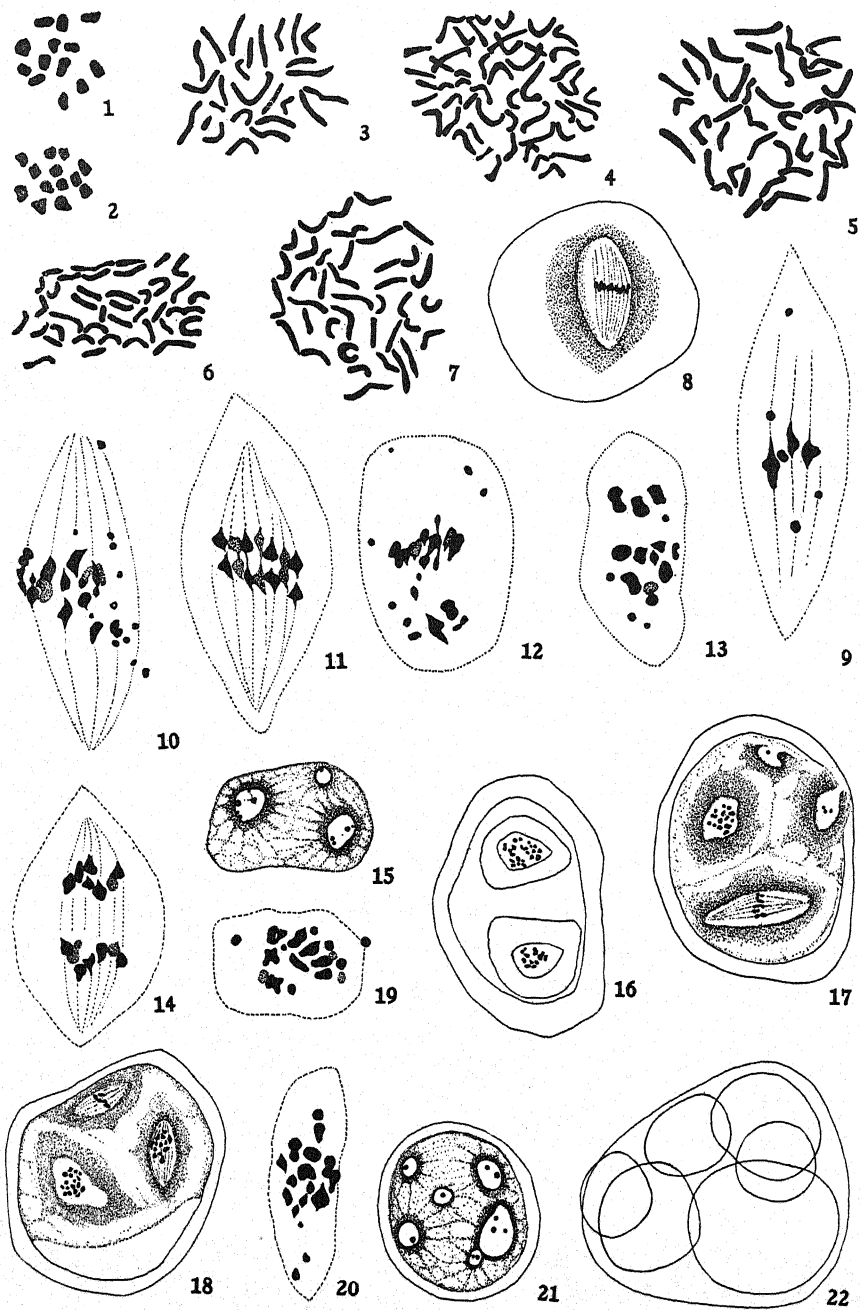
The haploid chromosome number for *G. hirsutum* was not determined from microsporocytes in this work.

### Chromosome behavior in $F_1$ hybrids

The somatic chromosomes of the three hybrid plants A, B, and C have been studied in root tips. These contained 39 chromosomes, which is the summation of the haploid 26 from the American parent and the haploid 13 from the Asiatic parent. There is no difficulty in making counts, because the nuclear plate is nearly as large as that of American cotton and the chromosomes are not too crowded.

The following description of chromosome behavior in microsporogenesis was drawn chiefly from observations on hybrid A, which was the result of a cross using *G. nanking* as the female and *G. hirsutum* as the male parent. The chromosome behavior in meiosis in the other two hybrid plants, B and C, was similar to that described later in this paper.

The chromosome behavior before the first metaphase has not yet been traced. At metaphase of the first division the chromosomes may all lie in the same equatorial plane (fig. 8), although in most cases some of the univalents remain away from this plane (fig. 9). In polar view the bivalents and univalents can be distinguished by their sizes; the bivalents are much larger than the univalents. This difference is most clearly seen in the side view, where the univalents are small and round and the bivalents large and irregular, as if they were pulled by the spindle fiber attachment (fig. 9). The number of bivalents is generally 13 (fig. 10). The greatest irregularity comes at the anaphase, when the bivalents and univalents are scattered throughout the spindle. The univalents are distributed to the poles at random without splitting. Some of the bivalents disjoin, but others, often a large number, move toward the poles without any sign of disjunction (fig. 10); in normal plants the disjunction at anaphase is quite regular, as shown in figure 11. In a later anaphase stage, when two groups of chromosomes are pulling apart, the univalents and non-disjoined bivalents are clearly seen in each group



FIGS. 1-22.—Legend on opposite page.

and the two groups often contain different numbers of chromosomes (figs. 12, 13); in normal plants no such irregularity is seen (fig. 14). Some of the scattered chromosomes are excluded from the two daughter nuclei and form micronuclei at the end of the first division (fig. 15). There may be one to several micronuclei in interkinesis.

At metaphase of the second division the two equatorial plates frequently show different chromosome numbers (fig. 16). This is the expected result of the unequal distribution of chromosomes observed at the first division. The large bivalents and the small univalents are also clearly seen in the equatorial plates (figs. 16, 19). At this stage there are generally two major spindles; the spindles of the micronuclei are not clear (fig. 17). In other cases, however, a third spindle is conspicuous (fig. 18). In side view of the second metaphase all the chromosomes may be arranged normally in an

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FIGS. 1-22.\*—Fig. 1, first metaphase in microsporocyte of *G. nanking*, polar view, showing 13 chromosomes;  $\times 1650$ . Fig. 2, second metaphase in same, showing 13 chromosomes;  $\times 1650$ . Fig. 3, somatic metaphase of *G. nanking*, showing 26 chromosomes;  $\times 2500$ . Fig. 4, somatic metaphase of *G. hirsutum*, showing 52 chromosomes;  $\times 2500$ . Fig. 5, somatic metaphase of *G. nanking* $\times$ *G. hirsutum*, showing 39 chromosomes;  $\times 2500$ . Figs. 6, 7, same, showing 39 chromosomes;  $\times 2500$ .

FIGS. 8-22 (with the exception of figs. 11 and 14) are of microsporocytes from one hybrid plant, *G. nanking* $\times$ *G. hirsutum*. Fig. 8, first metaphase, side view, showing regular arrangement of chromosomes in equatorial plane;  $\times 650$ . Fig. 9, same, showing bivalents and univalents (bivalents are in equatorial plane but some of the univalents are not showing on this plane);  $\times 1650$ . Fig. 10, early anaphase of first division, showing 13 bivalents and 13 univalents; also disjunction and non-disjunction of bivalents and irregular distribution of univalents;  $\times 1650$ . Fig. 11, early anaphase from a normal *G. nanking* plant, showing normal disjunction of bivalents;  $\times 1650$ . Figs. 12, 13, late anaphases in hybrid, showing non-disjoined bivalents going toward each pole;  $\times 1650$ . Fig. 14, late anaphase from a normal *G. nanking* plant, showing normal distribution of chromosomes;  $\times 1650$ . Fig. 15, interkinesis in hybrid, showing micronucleus in addition to two daughter nuclei;  $\times 650$ . Fig. 16, second metaphase, polar view, showing different chromosome numbers in two equatorial planes;  $\times 650$ . Fig. 17, second metaphase, showing one major spindle in side view, another in polar view, and two micronuclei in which spindle is not distinct;  $\times 650$ . Fig. 18, second metaphase, showing third spindle with four chromosomes in addition to two major spindles;  $\times 650$ . Figs. 19, 20, taken from same microsporocyte: fig. 19, second metaphase, polar view, showing different sizes of chromosomes. Larger ones are most probably non-disjoined bivalents from first division; fig. 20, early second anaphase, showing non-disjoined bivalents. Fig. 21, abnormal "quartet" containing 6 nuclei of different sizes. Fig. 22, 6 unequal-sized daughter cells formed from one microsporocyte.

\* Figures drawn with aid of a camera lucida and reduced to one-half.



equatorial plate (fig. 17). At the second anaphase the distribution of chromosomes is also irregular, the chromosomes being scattered throughout the spindle. The non-disjoined bivalents may disjoin at this time or still remain intact (fig. 20). As a result of such irregular distribution of chromosomes, there are often more than four nuclei formed from one spore mother cell. These may be unequal in size owing to differences in chromosome number. The micronuclei are the result of chromosome elimination in the first or second division (figs. 21, 22). The pollen grains containing abnormal chromosome complements probably are largely, if not wholly, abortive.

A point to be emphasized here is the non-disjunction of bivalents in the first and second divisions. NAKATOMI (18) noted that in the first division "occasionally there was found one or more bivalents located in the cytoplasm without division," and that "in most cells bivalent chromosomes which were not divided in the first division were found even in the second division." The evidence of the present investigation substantiates NAKATOMI's interpretation. LONGLEY did not mention the non-disjunction of bivalents and even gave evidence for the splitting of univalents in the first division.

The distribution of chromosomes in the second anaphase is still not fully described. NAKATOMI stated: "Most of the univalent chromosomes of each group and bivalent chromosomes were divided in homoeotypic division and the split halves of each chromosome were separated toward different poles, but some were not divided and left as dyad chromosomes." He did not make clear whether the word "some" referred to the bivalents or to the univalents. LONGLEY did not mention the chromosome behavior in the second division. Irregular quartets and abortive pollen grains were also reported in the works of NAKATOMI and LONGLEY.

### Summary

1. One thousand seven hundred and eight crosses were made between Asiatic and American cotton species and six hybrid plants were obtained.
2. Out of 1017 crosses in which Asiatic cottons were used as the female parent, one hybrid plant was produced, while in 691 crosses

using American cotton as the female, five hybrid plants were obtained. This would indicate that when crosses are made between Asiatic and American cotton species better success is obtained when American cotton is used as the female parent than when Asiatic cotton is so used. Since American cotton has 52 somatic chromosomes while Asiatic cotton has 26, this agrees with the general observation that crosses using the species with the higher chromosome number as the female are more successful.

3. Studies of pollen tube growth suggest that the low percentage of success in Asiatic-American species crosses is due not to the slowness of foreign pollen tube growth, but probably to an actual incompatibility of gametes.

4. The  $F_1$  hybrid plants obtained showed considerable hybrid vigor, especially in the height of the plant. The reciprocal crosses did not seem to show the same degree of vigor, but the evidence is not conclusive.

5. In the hybrid plants some characters were like those of the American parent, others were like those of the Asiatic parent, and still others were intermediate. The  $F_1$  plants could be identified by these mixed Asiatic and American characters.

6. The  $F_1$  plants were completely sterile when selfed. When pollinated with pollen of the parental species, one fruit with one seed was obtained but the seed did not grow. When the pollen grains were placed on the stigma of the American species some seeds were formed. These seeds gave rise to plants identical with the American parent. These plants were most probably the results of contamination.

7. The pollen grains of the  $F_1$  hybrids were very irregular in size and did not burst when mounted in water. They were mostly, if not all, abortive. The ovules were also abortive, as evidenced by their shrunken appearance.

8. The somatic chromosome number of *G. nanking* is 26 and that of *G. hirsutum* is 52. The haploid chromosome number of *G. nanking* is 13.

9. The somatic chromosome number of the hybrid plants is 39. This is the sum of 26 from the American and 13 from the Asiatic

parent. The knowledge of chromosome morphology is at present not sufficient to enable one to distinguish the chromosomes derived from the two parents.

10. Meiosis in the microsporocytes of the  $F_1$  hybrids was found to be irregular. In the first division metaphase, 13 bivalents and 13 univalents were observed. The univalents were distributed at random and part of the bivalents disjoined at the first division. The non-disjunction of bivalents was often observed, the entire bivalent moving to one pole. Some chromosomes were eliminated at the first division and formed micronuclei. In the second division more than two spindles were often observed. The chromosome distribution was also irregular at this division. There are thus formed more than four nuclei which are unequal in size and lead to the formation of irregular sized and abortive pollen grains.

11. Judging from the present status of our knowledge, the possibility of making fertile hybrids between Asiatic and American cotton species is remote unless the chromosome difference between them is removed in some way before crossing.

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# COMPARATIVE STUDY OF THE DEVELOPING AND ABORTING FRUITS OF PRUNUS PERSICA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 459

THOMAS J. HARROLD

(WITH TWENTY-SEVEN FIGURES)

## Introduction

A large percentage of the fruits of the peach fall from the trees before maturity, and this failure in development is a major problem among the growers of pome and stone fruits. The problem has been studied in considerable detail with the plum, the sour cherry, and the sweet cherry by DORSEY (7), BRADBURY (5), and TUKEY (12). With the exception of the work of DETJEN (6) and CONNORS (4), the peach has apparently been overlooked in this phase of the sterility problem.

The investigation covered the following points: (1) the situation in the peach as compared with the facts of abortion already known for the plum and cherry; (2) the careful comparison of developing and aborting fruits to aid in the discovery of the fundamental causes of this type of abortion; (3) the possible relation of the three growth periods of the peach, also recognized by other workers, to abortion and subsequent dropping of fruits.

## Materials and methods

The Carman variety of peach (*Prunus persica* Sieb. & Zucc.) was used in this study. Material was collected in the College orchard at Athens, Georgia, during the season of 1933. The first collections were made about one week before full bloom, thereafter daily for a 30-day period, and then at intervals of two or three days until about three weeks before maturity. This material was of two sorts, that which was expected to develop and that which would probably not do so. Material of the developing series was secured by removing certain branches, which were taken to the laboratory and placed in water,

and then the pistils were dropped into fixing solution. Aborting material was collected on the tree as soon as it could be recognized, and by catching falling fruits in a net after jarring the tree with a padded pole. The material, both developing and aborting, was prepared by cutting away portions of the pericarp; in later stages by removing the seeds in order that fixation might be facilitated. A modification of Nawaschin's solution (BEAL 2) and formalin-acetic-alcohol were used.

Most of the sections were cut at 10  $\mu$ , and stained in iron-alum haematoxylin or Flemming's triple stain. Measurements were made from fresh and from fixed material. The figures of the diagram in figure 1 represent in each case the average of five to twenty measurements.

Determination of total abortion was made by subtracting from the number of blossoms on certain limbs the number of fruits remaining on the same limbs after the third drop.

### Microscopic study

The Carman variety of peach used in this experiment dropped approximately 70 per cent of the flowers and fruits during the season of 1933 (table I). The fall of flowers and fruits occurs in waves which are designated as the first, second, and third drops. Each wave of dropping may be differentiated according to time of occurrence and by morphological characters.

Assuming that the figures of table I may be applied to the entire orchard, it is evident that pollination is not a problem.

Each wave of dropping consists of flowers or fruits which proceed normally in development until a short time before falling. This is clearly explained by BRADBURY (5), who divides the developing and aborting fruits into three groups: "Group I . . . includes two lots: (a) those aborting fruits which will fall in the first drop and (b) the corresponding developing fruits, that is, fruits which are developing while the former are aborting. Group II is composed of fruits which were classed as developing fruits in Group I, but have become divisible into two lots: (a) aborting fruits which will fall in the second drop and (b) developing fruits. Similarly, Group III is made up of

fruits classed as developing fruits in Group II, but have become separable into two lots: (a) aborting fruits which will fall in the third drop and (b) developing fruits."

It was possible in this study to recognize aborting fruits several days before their fall.

#### FIRST DROP

The first wave of peach drops occurs during approximately a 3-week period beginning one to three days before full bloom. This drop consists of buds and of flowers with and without petals, con-

TABLE I  
CARMAN VARIETY; 1933

	NUMBER OF BRANCHES	NUMBER OF FLOWERS AT FULL BLOOM	NUMBER OF FRUIT SET 2 WEEKS AFTER THIRD DROP	PERCENTAGE OF FLOWERS SETTING FRUIT
Open pollinated.....	8	1153	378	31.9
Flowers brushed with pollen, not bagged.....	6	991	301	30.3

taining shriveled pistils in some cases and plump pistils in others. Pericarp enlargement has not proceeded far enough to cause the shedding of the calyx cup. Fruits which develop lose the calyx cup about the middle of the first drop period (figs. 1, 2). Styles are present in both the dropped and the developing fruits. Some have two shriveled ovules as compared with the one plump ovule in all the developing fruits.

#### SECOND DROP

This wave of dropping occurs during approximately a 7-day period about the fifth week after bloom. It takes place during the middle of the first period of rapid development of the pericarp and integuments (fig. 1). Dropped fruits measure from 8 to 14 mm. in length, while the developing fruits measure 20 to 27 mm. (fig. 3). Color differences are apparent, the dropped fruits differing from developing fruits in being yellowish green as compared with the darker green of the latter. Both types are devoid of calyx cups.



## THIRD DROP

This wave occurs during approximately a 7-day period about the seventh week after bloom. These fruits vary widely in size, measuring 25 to 30 mm. compared with an average length of 37 mm. in the developing fruits (figs. 1, 4). The flaccid and yellow-green appearance which characterized the second drop is also evident. The integuments have attained nearly the size found in mature fruits.

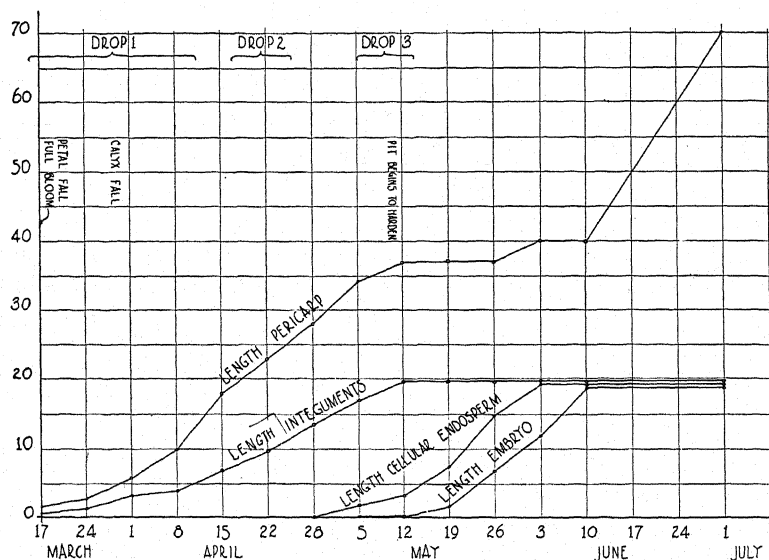


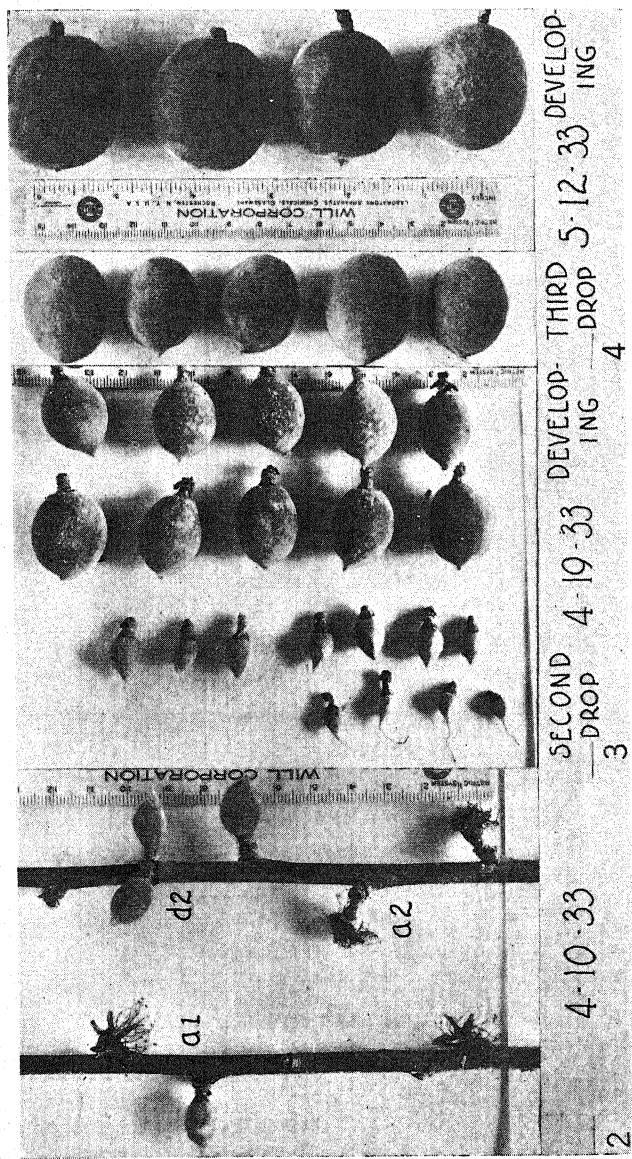
FIG. 1.—Diagram showing development of pericarp, integuments, cellular endosperm, and embryo in relation to time of drop.

## Microscopic study of normal development

Correlation of parts of developing fruits with time of development is given in figure 1. This includes measurements of pericarp, integuments, cellular endosperm, and embryo. The diagram also shows the relation of each of the three drops to the developing fruits.

## PERICARP

Enlargement of the pericarp of the peach does not proceed uniformly, but is divided into three stages (fig. 1). This has previously been recognized in the peach by CONNORS (3), DORSEY and Mc-



FIGS. 2-4.—Fig. 2, branch about the end of the first drop: *a1*, aborting first drop; *a2*, aborting second drop; *d2*, developing fruit. Fig. 3, condition during middle of second drop, showing aborting and developing fruits. Fig. 4, condition at about the end of the third drop, showing aborting and developing fruits.

MUNN (8), and LILLELAND (10); and in the sweet cherry by TUKEY (12). During the first period of eight weeks, the pericarp increased in length from 1.7 to about 37 mm., followed by a second period of four weeks in which the rate of development of the pericarp was retarded. The third period of three weeks is characterized by a final rapid enlargement of the pericarp, ending at maturity.

Pericarp enlargement is accounted for by ADDOMS, NIGHTINGALE, and BLAKE (1) as being associated with cell division during the first 30 days and subsequently with cell expansion.

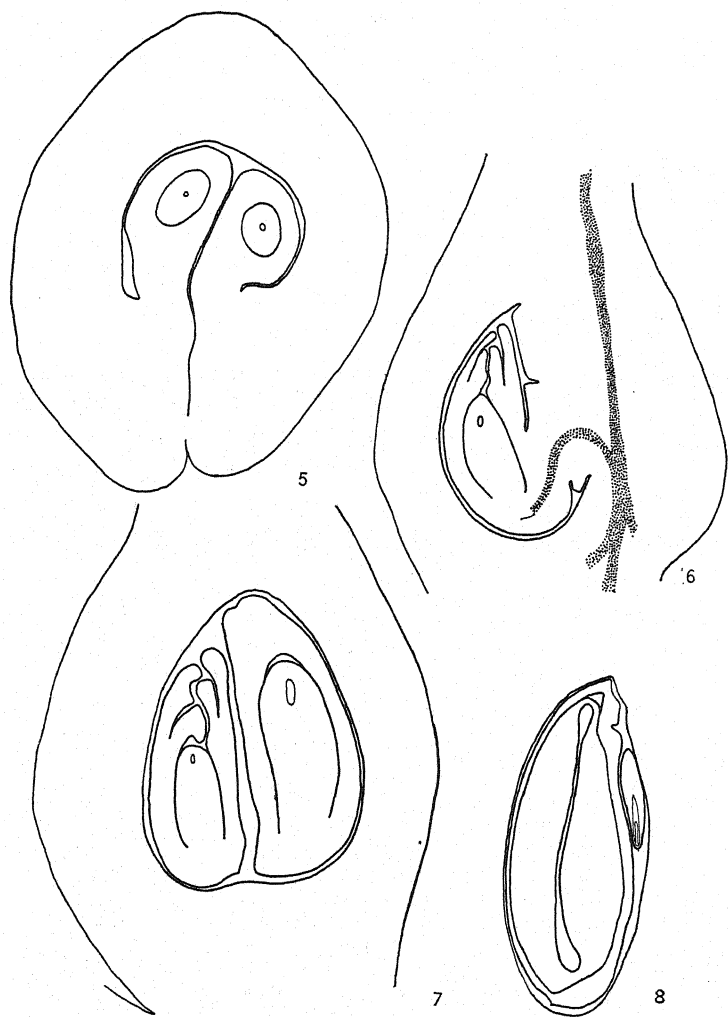
During the second period of growth (fig. 1), hardening of the inner wall of the pericarp takes place, forming the "pit."

#### NUCELLUS AND INTEGUMENTS

Two anatropous ovules are differentiated within a single carpel (figs. 5, 6). These two ovules develop equally until about three days before full bloom, when one of them gains the ascendancy and the other ceases to develop, so that at least a day before full bloom the latter may be recognized by its smaller size (fig. 7). The non-functional ovule is first recognized in stained sections by a peculiar transparency of the nucellus, while at the same time the gametophyte within appears normal. Degeneration involves shrinkage of the nucellus and a loss of characteristic organization by the gametophyte. The nucleoli of the latter apparently persist longer than do the other parts. These findings agree with those reported for the sour cherry (5).

The condition of a non-functional ovule at the time of full bloom is illustrated in figure 13. The latter is from the same carpel that contains the functional ovule shown in figure 25. Cessation of development of the non-functional ovule may occur at any point between the megaspore mother cell stage and the various stages in development of the gametophyte. Development of the functional ovule proceeds rapidly after full bloom, so that differences between the functional and the non-functional ovule become increasingly apparent. Figure 8 represents the relative development of the two ovules two weeks after fertilization.

The enlargement of the seed parallels the development of the pericarp during the first period of growth, so that by the close of the



FIGS. 5-8.\*—Fig. 5, transverse section of pistil at time of full bloom;  $\times 135$ . Fig. 6, longitudinal section of pistil at time of full bloom;  $\times 135$ . Fig. 7, functional and non-functional ovules at time of full bloom;  $\times 135$ . Fig. 8, relative development of two ovules two weeks after fertilization, showing elongation of embryo sac.;  $\times 45$ .

\* All drawings were made with the aid of an Abbé camera lucida.

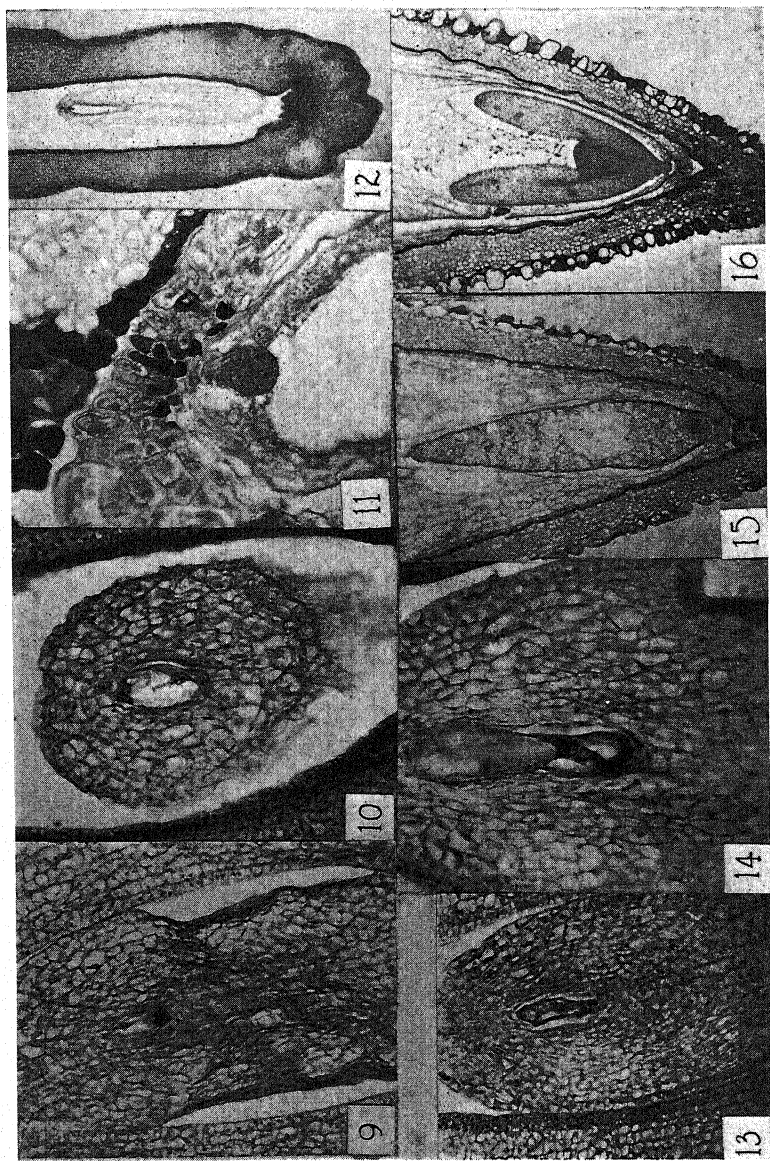
8-week period after bloom the integuments have attained a final and maximum size of about 20 mm. (fig. 1). The early maturation of the integuments has been recognized by many workers, including PÉCHOUTRE (11), TUKEY (12), DORSEY and McMUNN (8), and CONNORS (3). The maximum size of the integuments is reached about two weeks after cellular endosperm appears and is approximately coincident with an appreciable enlargement of the embryo.

The two integuments of the peach are distinct for only about one-fourth of their length (fig. 6).

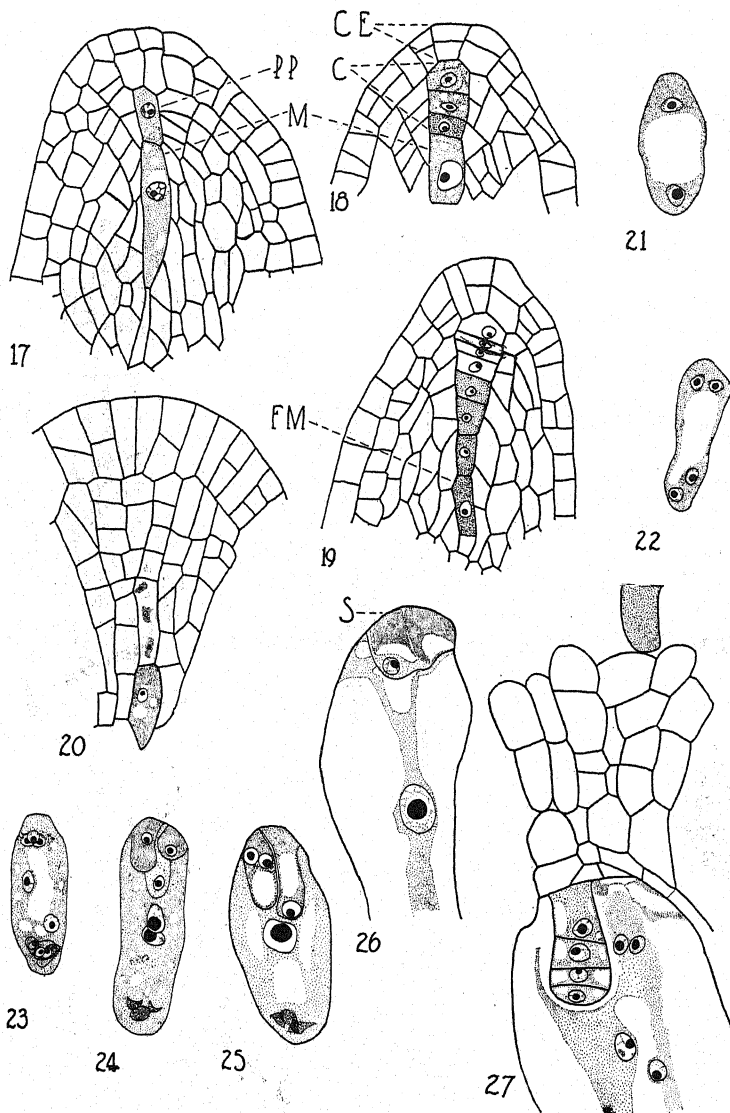
#### MEGAGAMETOPHYTE

The megaspore mother cell (fig. 18) is imbedded in the nucellus, being covered with three or four parietal cells and two or three layers of cells derived from the epidermis of the nucellus at the micropylar end (coiffé épidermique and calotte respectively of PÉCHOUTRE 11). Reduction divisions resulting in a linear tetrad of megaspores (fig. 19) occur a few days before bloom. The chalazal megaspore develops and the remaining megaspores rapidly disintegrate (fig. 20). The three divisions of the functional megaspore (figs. 21, 22) follow in rapid succession, so that a day or two before full bloom the megagametophyte (fig. 23) is in the 8-nucleate stage. Maturation and migration of the two polar nuclei to the middle of the megagametophyte (fig. 24) is practically coincident with full bloom. The two polar nuclei unite (fig. 25) during the period between full bloom and fertilization. The three antipodal nuclei are short-lived (fig. 25), being well along toward disintegration at the time of polar nuclear fusion. The synergids persist for about a week after fertilization.

The embryo sac begins to elongate following the union of the polar nuclei, doubles in length by the time of fertilization, and within two weeks extends almost to the chalaza (fig. 8). The embryo sac is characteristically swollen at the chalazal end, and owing to its narrow connecting canal forms a more or less dumb-bell shaped structure. TUKEY (12) reports the membrane surrounding the elongated sac to be continuous and persistent. According to BRADBURY (5) and DORSEY (7), when fertilization is prevented elongation of the embryo sac takes place, extending as much as two-thirds of the distance to the chalaza.



FIGS. 9-16.—Fig. 9, portion of ovule from aborting pistil of first drop, prior to full bloom;  $\times 400$ . Fig. 10, portion of ovule from aborting pistil of first drop, after full bloom;  $\times 400$ . Fig. 11, embryo, endosperm, nucellus, and integument development in a developing fruit at time of second drop;  $\times 400$ . Fig. 12, embryo, endosperm, nucellus, and integument development in a developing fruit of second drop;  $\times 63$ . Fig. 13, portion of non-functional ovule at time of full bloom (same carpel as in fig. 25);  $\times 400$ . Fig. 14, portion of ovule showing primary endosperm nucleus (zygote in adjoining section) of fruit of first drop, apparently "healthy." Note shrinkage of nucellus at chalazal end;  $\times 400$ . Fig. 15, portion of ovule showing early cellular endosperm, 7 weeks after full bloom;  $\times 63$ . Fig. 16, portion of developing fruit at time of third drop. Note embryo and cellular endosperm;  $\times 63$ .



FIGS. 17-27.—Fig. 17, portion of nucellus of ovule prior to full bloom, showing primary parietal cell (*pp*) and megaspore mother cell (*m*). Fig. 18, portion of nucellus of ovule prior to full bloom, showing coiffé épidermique (*ce*), three parietal cells (*c*), and megaspore mother cell (*m*). Fig. 19, portion of the nucellus showing four megaspores and functioning megaspore (*fm*). Fig. 20, portion of nucellus showing functioning megaspore and three disintegrating megaspores. Fig. 21, megagametophyte at 2-nucleate stage. Fig. 22, same at 4-nucleate stage. Fig. 23, same at 8-nucleate stage. Fig. 24, megagametophyte at time of full bloom. Note disintegration of antipodals. Fig. 25, megagametophyte just prior to fertilization. Note fusion of polar nuclei and their proximity to megagamete. Fig. 26, embryo sac following fertilization. Note disintegrating synergid, zygote, and primary endosperm nucleus. Fig. 27, portion of nucellus two weeks after fertilization. Note remains of pollen tube, 4-celled embryo, and numerous free nuclei of endosperm. All  $\times 1860$ .

## FERTILIZATION

Double fertilization as described by BRADBURY (5) and reported by TUKEY (12) is found also in the peach. Evidence of fertilization was found in material in which the style had begun to fade, which was collected four days after full bloom. Prior to fertilization, the megagamete and the fused polar nucleus take positions close together (fig. 25). For a short period after fertilization the one-nucleate embryo and one-nucleate endosperm remain close together; then begins a gradual movement of the primary endosperm nucleus down the embryo sac toward the chalaza.

## ENDOSPERM

Following fertilization, the endosperm remains uninucleate for a period of nine or ten days (fig. 26). The first division of the primary endosperm nucleus takes place at a point nearly one-half of the distance to the chalaza. Following the first division there occur in rapid succession numerous divisions of endosperm nuclei, so that the embryo sac is soon lined with a peripheral layer of free-nuclear endosperm (fig. 27).

This coenocytic condition persists until about the end of the sixth week after bloom. Walls begin to appear at that time at the micropylar end of the embryo sac. Development of cellular endosperm is rapid, so that during the following five weeks it comes to extend to the chalaza. This development takes place during the period of retarded pericarp enlargement (fig. 1).

## EMBRYO

The zygote nucleus does not divide until about 12 days after fertilization, at which time there have been many divisions of the endosperm nuclei. Embryonal development is extremely slow until about a week before the beginning of the period of retarded pericarp growth and until after considerable cellular endosperm has been formed (fig. 15). The early retardation of the embryo has been noted by CONNORS (3), and by DORSEY and McMUNN (8); and by TUKEY (12) in the sweet cherry. Acceleration of the embryonic growth begins about the time that the cellular endosperm has reached one-third to



one-half the length of the nucellus. Cotyledons (fig. 16) are differentiated and the embryo quickly extends almost to the chalaza, digesting cellular endosperm and nucellus before it, and completing its elongation approximately 12 weeks after bloom (fig. 1). Following the attainment of full size of the embryo, the final 3-week period of pericarpic enlargement occurs.

### Microscopic study of aborting fruits

#### FIRST DROP

The buds which fall before, during, and after full bloom have pistils in various stages of development and degeneration, which have apparently proceeded normally up to a point between the megaspore mother cell and the early gametophyte stages. Growth then stops and degeneration begins. Buds which fall in the first drop contain pistils in which both ovules have ceased to develop. The degeneration of the second ovule closely resembles that of the first. This check in development of both ovules before opening of the flower is recognized in the plum by DORSEY (7) and in the sour cherry by BRADBURY (5).

Flowers which fall with expanded petals contain ovules which have proceeded still further in development, usually being found in the two-, four-, or eight-nucleate stages of the megagametophyte. These megagametophytes evidence nuclear and cytoplasmic degeneration such as has been described for the non-functional ovule.

Flowers without petals also fall in the first drop. As a rule ovules of these pistils indicate that development has proceeded to a still further degree than that which has been described for those with petals. In this group are found ovules in which degeneration apparently began at the mature megagametophyte stage (fig. 10). Other ovules in this group give evidence of fertilization having taken place (fig. 14). In contrast to this, DORSEY (7) accounts for the entire drop on the basis of pistil abortion. The conditions shown in figures 10 and 14 are described by BRADBURY as being found in the second drop, in cases in which an embryo is not present. In certain cases (fig. 14) there is evidence of a shrinking of the nucellus at the chalazal end, while the embryo sac is apparently normal.

## SECOND DROP

Fruits which fall in the second drop are among those which were considered as developing during the course of the first drop. Growth has proceeded to a further degree, the general relations of which are shown in figure 1. This drop occurs during the time of free-nuclear endosperm and early embryonal stages of developing fruits. The condition of embryo and adjacent endosperm is shown in figure 11, while the conditions of the corresponding drop is shown in figure 12. There is apparently no lack of development of integuments shown in the dropped fruits, the chief differences between the developing and aborting fruits being found in the nucellus, endosperm, and embryo.

DORSEY (7) reports this drop to be a result of lack of fertilization, while BRADBURY, on the contrary, finds that in some cases fruits of the second drop do have embryos and in other cases do not. Fruits in which pollination was prevented were found by both DORSEY and BRADBURY to fall in the first and second drops.

## THIRD DROP

The embryos from fruits which fall in the third drop exhibit no apparent structural differences from those of developing fruits (fig. 16). DORSEY (7), BRADBURY (5), and TUKEY (12) report that fruits which fall in the third drop contain apparently normal embryos which, for some reason, have ceased development, size differences being the only observable ones between developing and aborting embryos. Fallen fruits contain seeds in which the integuments have begun to turn brown at the chalazal end, in contrast to no browning of those of seeds of developing fruits. This condition of browning is reported by DORSEY (7) as being evident both in the second and the third drops of the plum.

## Discussion

The only apparent difference between aborting fruits and developing fruits is that growth has stopped in one and has continued in the other. Thus differences between developing and aborting fruits at the time of falling exhibit mainly degrees of development, and in some cases degeneration of normal parts. At the exact moment of cessation of development, there should be no structural differences

between fruits that are to develop to maturity and those that are to abort. In the first drop is found cessation of development at stages leading up to the gametophyte, also of gametophytes and of zygote and primary endosperm nucleus; and in the second and third drops a cessation of development of embryo and endosperm, which agrees in the main with the report of BRADBURY (5). This differs from the report of DORSEY (7) in that the first drop includes more than only "aborted pistils" and that the second drop results from more than "lack of fertilization," but agrees in regard to the third drop.

Granting the fact that development proceeds normally until the time of abortion, a diagram (fig. 1) may aid in the prediction of the approximate time at which development ceases, and the degree of development of the parts of each dropped fruit. Thus if the fruits of the second drop measure 8 to 14 mm. in length, it is reasonable to expect that growth stopped at about the time that most of the fruits had attained this measurement, which the chart shows to be approximately April 6-13. It is inferred also that these fruits fell approximately April 17-26. According to the diagram, the fruits which developed until April 6-13 possessed free-nuclear endosperm and a slight degree of embryonal development. The material shows this to be true. Likewise in the third drop the chart predicts cellular endosperm and additional embryonal development, which also is confirmed by the material.

TUKEY (13) has demonstrated that if the embryos from an early variety of sweet cherry, which does not produce viable seed, are placed under the proper conditions of artificial culture they continue to develop. As described by him, the embryos of the fruits which were checked in development before maturity of the embryo were in many ways similar in condition to the embryos contained in fruits of the third drop of the peach, the chief difference being that TUKEY (12, 13) was working with aborted embryos of developing fruits and not aborted embryos of dropping fruits. If it were possible to demonstrate that embryos of fruits falling in the third drop could be artificially cultured, then it would appear that the cause of the third drop was not due to the fact that the embryos ceased to develop, but rather as a result of some other factor.

DORSEY (7) observed in the second drop of the plum that the integuments begin to turn brown at the chalazal end, and that there is a shrinkage of the nucellus, before the pistil falls, and in the third drop that the nucellus is shriveled and "early browning of the seed coats soon follows suppression of the embryo." BRADBURY (5) found associated with cessation of development in each of the three drops a shrinking of the nucellus. TUKEY (12) inferred that inasmuch as in the early-ripening non-viable seed varieties of sweet cherry the embryos are often proportionately larger than the nucellus and integuments (a condition not found in viable seed varieties), perhaps "a check in the development of integuments and nucellus precedes a check in the development of the embryo."

Cases such as shown in figure 14 represent a situation in which apparently degeneration of the nucellus in the region of the chalaza precedes any change from normal in the embryo sac. These observations lead toward the idea that degeneration, and possibly cessation of development in fruits which will drop, begins at the chalazal end of the ovule. The seeds of fruits which fall in the second and third drops are brown at the chalazal end, the browning extending toward the micropylar end as degeneration proceeds. These observations suggest that in the course of abortion development stops first in the region of the chalaza and extends from there to the other parts of the seed and fruit. Such a disorder in the chalazal region would disarrange the vascular system, and might mean that development of the entire fruit stops because of a failure of conduction. This suggests that possibly the fruit falls, not because the embryo aborts but because these events are occasioned by an upset of the vascular system in the region of the chalaza.

### Summary

1. The development of the megagametophyte and fruit of the peach agrees with facts established for other members of the genus *Prunus*.

2. The dropping of pistils occurs in three distinct waves during the first growth period, and each wave bears a constant relation to the developing pistils.

3. The aborting fruits differ from developing fruits mainly in degree of development of normal parts.

4. The possibility is indicated that a disorder in the region of the chalaza may occur prior to cessation of development of gametophyte or of embryo, resulting in subsequent degeneration of the seed and fruit.

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# CYTOLOGICAL EFFECTS OF A GENE IN DATURA WHICH CAUSES DYAD FORMATION IN SPOROGENESIS

SOPHIA SATINA AND A. F. BLAKESLEE

(WITH THIRTY FIGURES)

## Introduction

Several genes responsible for disturbances connected with the reduction division have been found in plants from nature (1, 2, 3, 4). In some of the cases reported, only the pollen mother cells were affected. Thus BEADLE (2) found a failure of cytokinesis in maize during meiosis, but this was confined to the microsporocytes. In the other cases described (1, 3) a recessive gene caused disturbances in the meiosis of both mega- and microsporocytes.

The meiotic irregularities discussed in the present paper were discovered by Mr. S. HOROWITZ in the summer of 1931, during his brief stay at the Department of Genetics while in this country as a Guggenheim Fellow. In a group of  $F_2$  *Datura* cultures from radium-treated pollen, HOROWITZ found one segregating culture of 35 plants which had eight individuals with the young microspores in dyads instead of in tetrads. The pollen grains (mature microspores) of such plants were twice the size of those of normal diploids. As was proved later, this formation of dyads was due to a recessive gene which was named "dyad" (dy). Dyad plants were recognizable by external appearance, since they were more open in habit of growth.

The chromosomes of dyad plants were found to form 12 normal bivalents at the metaphase stage of the first meiotic division. In the pollen grains, however, there were 24 instead of the 12 chromosomes normally present. Since HOROWITZ was unable to carry further his study of the dyad type, the investigation was continued by the present writers.

The dyad gene has been continued through four generations by female backcrossing a dyad to a normal and later recovering dyad plants in the next generation by selfing the heterozygous parents.

When selfed, a  $2n$  dyad plant gives rise to  $4n$  dyads. The latter produces seed with difficulty, but a single malformed plant in the offspring had 72 chromosomes in somatic tissue, showing that this individual was  $6n$  in chromosomal constitution.

A later report will be made of the genetic location of the gene involved and of other matters relating to the breeding behavior. The present paper gives the results of a detailed cytological study of both mega- and microsporogenesis, and also of both female and male gametogenesis.

### Methods

The following fixatives were found by testing to be most satisfactory:

1. Lewitzky's fixative, consisting of 1% chromic acid (5 parts) and 10% formaldehyde (4 parts).
2. Modified Carnoy, consisting of 85 cc. of 70% alcohol, 5 cc. glacial acetic acid, and 5 cc. commercial formaldehyde.
3. Belling's iron acetocarmine.

Lewitzky's fixative gives the best results when it is used for studying details of early stages. This is true both for sectioned material and for smears. Modified Carnoy is generally good for all stages. Megasporogenesis and female gametogenesis had to be studied from sectioned material. Sections were cut from 5 to  $10\mu$  in thickness and stained with Haidenhain's iron haematoxylin. Microsporogenesis and male gametophytes were studied both from sectioned material and from smears. Smears were stained with Haidenhain's iron haematoxylin and destained with picric acid as recommended by TUAN (8). Frequent use of Belling's iron acetocarmine method also was made. For studying the mitotic division in the young microspores (pollen grains), chloral hydrate was added (roughly one drop of 10% solution of chloral hydrate to one drop of iron acetocarmine). This treatment cleared the cytoplasm from inclusions which otherwise obscured the chromosomes. All the drawings in this paper which represent stages in microsporogenesis and in male gametogenesis were from acetocarmine preparations, except figures 15 and 19, which were from material fixed in Lewitzky and Carnoy respectively.

## Observations

### MEGASPOROGENESIS

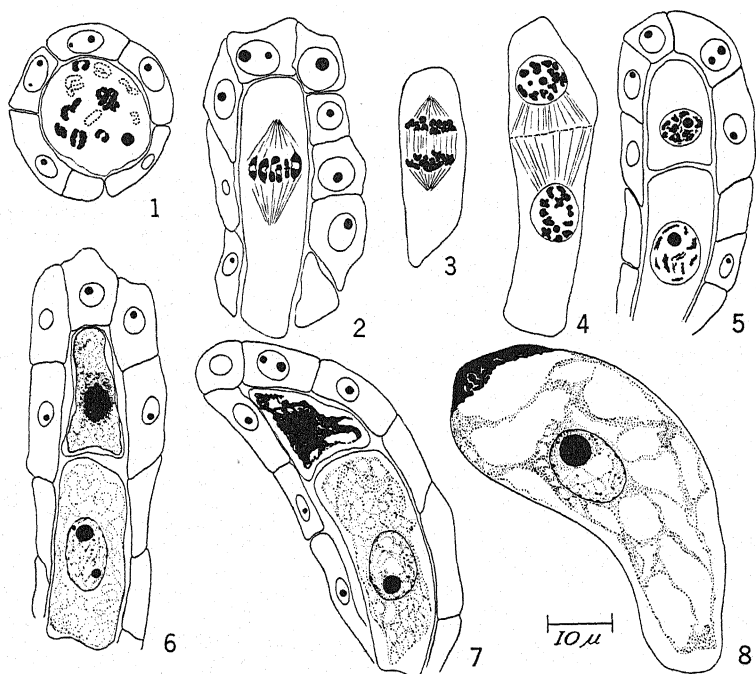
The megaspore mother cell which differentiates from the subepidermal cells of the nucellus is easily recognized at an early stage in the development of the ovule by its larger size and by its affinity for the stain. During prophase of the first meiotic division the size of this cell and of its nucleus increases considerably. The typical stages of early prophase were seen. Beginning with the diakinesis stage, a detailed study was made of the meiotic divisions of the megaspore mother cell.

Figure 1 shows the 12 bivalent pairs of chromosomes lying within the nuclear membrane. Figure 2 shows a later stage after the nuclear membrane has disappeared and the spindle has formed. Bivalent chromosomes lie in the equatorial plane. Chromosomes then pass to the poles during anaphase I (fig. 3). Telophase I is recognized by the formation of a nuclear membrane around each of the two groups of 12 split chromosomes (fig. 4). A cell plate begins to form in the middle of the spindle, as shown in figure 4, and apparently develops a cell wall which separates the two daughter nuclei as shown in figure 5. A comparison with corresponding stages of development in our standard Line 1 *Datura* shows that the first meiotic division in dyad plants does not differ apparently from that found in normal individuals. There is, however, a distinct difference in the stages following the first division. The second meiotic division is never found in dyad plants, except in occasional cells to be discussed later. Apparently the daughter nuclei formed as a result of the first meiotic division go into a resting stage after a gradual telophase transformation (fig. 5). This stage persists during the interval when the second division ordinarily takes place. As a result of the failure of the second division only two megaspores (dyad) are formed instead of the usual four (tetrad). The megaspore that lies at the micropylar end is not viable. Its nucleus and cytoplasm gradually disintegrate (figs. 6, 7), while the cell at the chalazal end, together with its nucleus, enlarges greatly (fig. 8). By further divisions the megaspore thus formed gives rise to the female gametophyte.



## FEMALE GAMETOGENESIS

In agreement with GUIGNARD (6), who described the development of the female gametophyte in *Datura laevis* (an inermis variety of *D. stramonium*), we find that the megaspore nucleus undergoes three successive mitotic divisions, resulting in the formation of an embryo

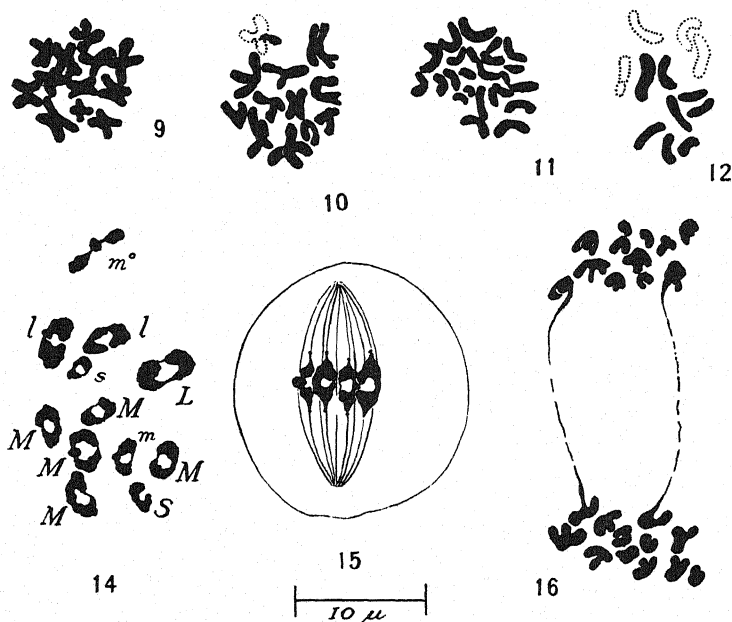


FIGS. 1-8.—Megasporogenesis in dyad plants of *Datura stramonium*. In figs. 3, 4 and 8 the cells of the nucellus are not shown: Fig. 1, 12 bivalents at diakinesis in megaspore mother cell. Fig. 2, metaphase I, six bivalents omitted. Fig. 3, anaphase I. Fig. 4, telophase I, two daughter nuclei each with 12 chromosomes within a nuclear membrane; formation of cell plate. Fig. 5, two megaspores (dyad), the lower with chromosomes indistinct in outline. Fig. 6, two megaspores; nucleus and cytoplasm of upper cell disintegrating; lower cell with resting nucleus. Fig. 7, later stage. Fig. 8, mature megaspore.  $\times 850$ .

sac which contains eight nuclei. In dyad plants the first division is the most interesting, since it furnishes evidence in support of the conclusion that the second meiotic division is lacking. Figures 9 and 10 show the chromosomes of the megaspore in the dyad plant at the metaphase stage of this first meiotic division. Twelve *pairs* of chro-

mosomes are present instead of the 12 *single* chromosomes seen in the megaspore of normal plants shown in figure 12. The gametophyte of dyad plants thus starts out with  $2n$  instead of with  $1n$  chromosomes.

It is known, both genetically and cytologically, that at the end of the first meiotic division in normal plants each chromosome is



FIGS. 9-12, 14-16.—Figs. 9-11, mitosis in megaspores of dyad plants. Fig. 12, mitosis in megaspore of first normal plant. Figs. 14-16, microsporogenesis in dyad plants: Fig. 9, metaphase of first mitotic division in megaspore showing 24 chromosomes lying in pairs. Fig. 10, similar stage with two chromosomes split. Fig. 11, metaphase of third mitotic division in megaspore showing 24 chromosomes. Fig. 12, metaphase of first mitotic division in normal megaspore showing 12 single chromosomes. Fig. 14, 12 bivalents at metaphase I in pollen mother cell. Fig. 15, metaphase I, eight bivalents omitted. Fig. 16, anaphase I, split chromosomes at each pole.  $\times 1700$ .

really double, the two chromatids being separated from each other during the anaphase stage of the second meiotic division. Since this second meiotic division is lacking in dyad plants, these double chromosomes have had no opportunity to separate into two groups. Twelve *double* chromosomes therefore are what one would expect at

the metaphase of the first division of the megaspore. In figure 10 some of the chromosomes show a secondary splitting in anticipation of the second division of the megaspore.

At the metaphase stage of the second and third divisions, each nucleus has 24 single chromosomes. Figure 11 shows the chromosomes of one of the four nuclei in the third division. Apparently the

paired condition of chromosomes seen at metaphase of the first mitotic division in the megaspore does not persist into later divisions.

After the third division eight nuclei are formed, each containing  $2n$  chromosomes. Cell membranes develop around six of these nuclei. The mature embryo sac contains at the micropylar end one egg cell and two synergids; the chalazal end has three antipodal cells which disintegrate very early. The two polar nuclei usually fuse near the egg cell. Figure 13 shows the beginning of disintegration of antipodal cells at an early stage when the polar nuclei are just beginning to move toward one another. The mature embryo sac of dyad

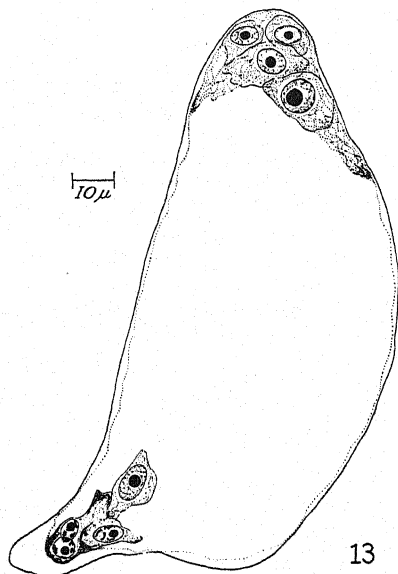


FIG. 13.—Female gametophyte in dyad plant shortly before fertilization. Egg cell, two synergids, and one polar nucleus at micropylar end of embryo sac; three disintegrating antipodal cells and one polar nucleus at chalazal end of embryo sac.  $\times 550$ .

plants is thus similar in structure to the embryo sac in a normal plant, except for the fact that each nucleus contains 24 instead of 12 chromosomes.

#### MICROSPOROGENESIS

The processes observed in pollen mother cells during microsporogenesis in dyad plants are essentially the same as those found in megasporogenesis. We have (a) an apparently normal first meiotic

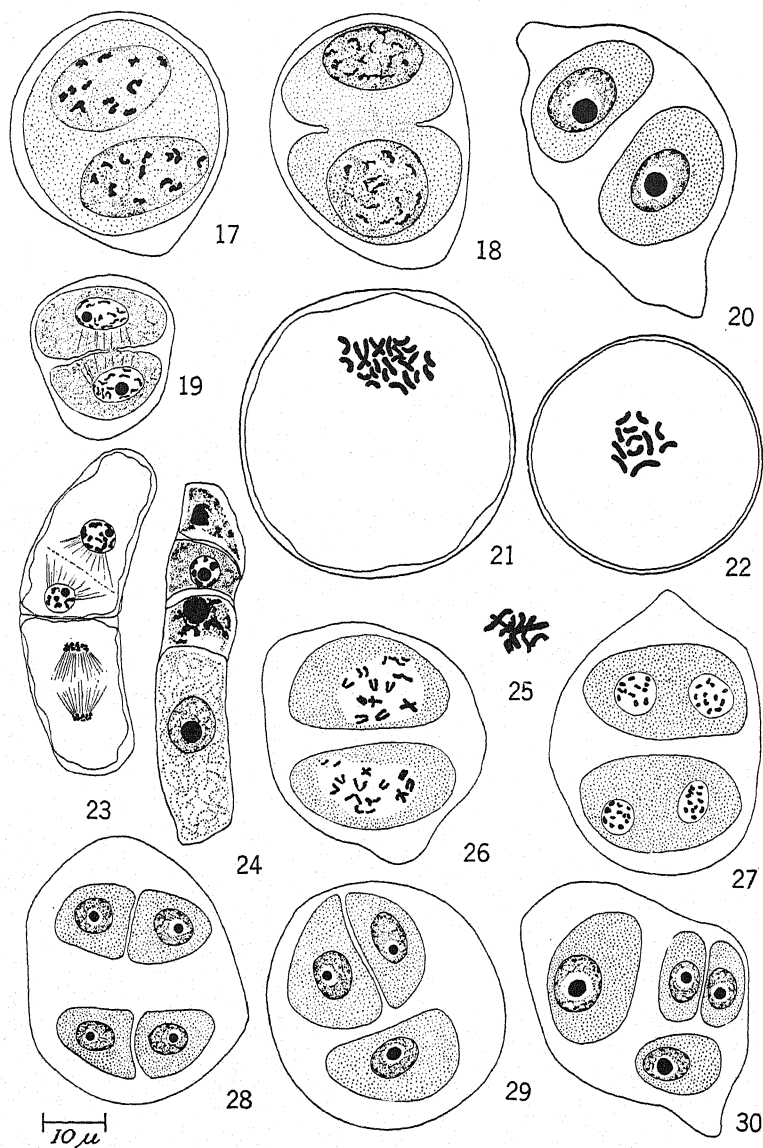
division; (b) a failure of the second meiotic division; (c) the formation of two microspores (dyads), instead of four (tetrads); and (d) the presence of 24 chromosomes in pollen grains. These conclusions, which were derived from a detailed study of microsporogenesis, confirm the earlier observations made by HOROWITZ.

Figure 14 shows metaphase of the first meiotic division in the pollen mother cell with 12 apparently normal bivalents in the equatorial plane. Figure 15 is a side view of the same stage and shows the spindle. After the separation of the bivalents in anaphase I, 12 split chromosomes are found at each pole of the spindle, as is shown in figure 16. At an early stage of telophase I the number of chromosomes can be counted (fig. 17). A nuclear membrane is formed around each group. As the telophasic transformation proceeds, the chromosomes in nuclei become slender, irregular, and indistinct in outline (fig. 18). In normal *Datura* species, interkinesis is of brief duration. The chromosomes are never completely lost from view. In dyad plants, however, "interkinesis" proceeds until a real resting stage is reached.

In *Datura* cytokinesis is simultaneous, that is, there is no division of the cytoplasm until after telophase II. No cell plates are formed on the spindles between the daughter nuclei. Cytokinesis begins with the formation of furrows which move in from the edges of the cell to divide the cytoplasm into four portions. Thus a tetrad of young microspores is formed within the pollen mother cell. In dyad plants, cytokinesis begins soon after the first telophase and before the nuclei have reached the resting stage. A furrow moves (fig. 18) in from the edges of the cell and bisects the pollen mother cell into a dyad of microspores. Figure 19 shows an advanced stage of cytokinesis although a few achromatic fibers are still present. By the time the nuclei have reached the resting stage, a thick cell wall has been formed between them (fig. 20). Later the two microspores thus formed slip out of the membrane of the pollen mother cell.

#### MALE GAMETOPHYTE

The development of the male gametophyte in dyad plants is quite normal, except for the number of chromosomes involved. As in nor-



FIGS. 17-30.—Figs. 17-20, microsporogenesis in dyad plants (continued). Fig. 21, mitosis in pollen grain in dyad plant. Fig. 22, same in normal plant. Figs. 23-25, exceptional cases in megasporogenesis and female gametogenesis in dyad plants. Figs. 26-30, same in microsporogenesis in dyad plants: Fig. 17, telophase I in pollen mother cell, two daughter nuclei each with 12 chromosomes within nuclear membrane. Fig. 18, further development; chromosomes indistinct in outline; beginning of cytokinesis by furrow at edge of cell. Fig. 19, advanced cytokinesis; pollen mother cell bisected into a dyad of microspores; few achromatic fibers still present. Fig. 20, microspores in dyad, with resting nuclei. Fig. 21, metaphase stage of mitotic division in pollen grain of dyad plant showing 24 chromosomes. Fig. 22, metaphase stage of mitotic division in pollen grain of normal plant showing 12 chromosomes. Fig. 23, completed second meiotic division. Fig. 24, four megaspores, three of which degenerate; nucleus of lower megaspore at resting stage. Fig. 25, female gametogenesis; metaphase of third mitotic division of nucleus showing 12 chromosomes. Fig. 26, second metaphase in a dyad. Fig. 27, second telophase. Fig. 28, tetrad of microspores. Fig. 29, triad of microspores. Fig. 30, tetrad of microspores of unequal size.  $\times 850$ .

mal *Datura* species, each young pollen grain has a single large nucleus in the middle of the cell and a dense cytoplasm. A thick exine develops gradually. Just before the pollen grain becomes opaque, owing to the development of starch, the nucleus, which now lies close to one edge of the cell, goes through a mitotic division. Figure 21 shows the metaphase stage of this division. Since there was no second meiotic division, each microspore of a dyad plant has 12 double chromosomes; that is, 24 chromosomes instead of the usual 12 single ones of normal plants shown in figure 22. In figure 21 there is a suggestion of pairing of sister chromosomes like those shown in the first meiotic division of the megaspore (figs. 8, 10). Around one of the daughter nuclei a membrane cuts out the generative cell, separating it from the other nucleus. This latter nucleus with the rest of the pollen grain becomes the vegetative cell of the male gametophyte.

#### EXCEPTIONAL CASES

It should be mentioned that a few megaspore mother cells develop in the same way as those of normal plants, in that both the first and the second meiotic divisions take place. (Figure 23 shows such a second division in a dyad plant.) As a result, four megaspores are formed. Three of these disintegrate (fig. 24) and the nucleus of the megaspore which remains viable has 12 single chromosomes, as is the case of normal *Datura* (fig. 12). The succeeding nuclei of the gametophyte will therefore have only 12 chromosomes. Figure 25, for example, shows the third mitotic division of one of these nuclei with 12 chromosomes from a dyad plant. The number of megaspore mother cells which showed second divisions varied considerably. In most of the buds examined no such cases were found. In other cases there must have been approximately 1 to 5 per cent of second divisions, judging from the number of second divisions actually observed or calculated from the presence of four megaspores.

The same exceptional occurrence of the second meiotic division has been observed during microsporogenesis. This division takes place either soon after telophase I, as in normal plants, or later, after a thick wall has separated the two dyads. As a result, four microspores are formed, each with 12 chromosomes as in normal plants

(figs. 26-28). Rarely one only of the two dyad cells undergoes a division, thus giving rise to three microspores (fig. 29), one with 24 and two with 12 chromosomes. In two cases a further, supernumerary division has been observed in one of these two microspores, which had resulted from a second division. Two microspores of half normal size (fig. 30) are thus formed.

The cytological observations can be checked in two ways: by the breeding behavior and by the sizes of the pollen grains. Dyad plants can be used as females, in crosses to normals, although the number of seeds set is much below average. The offspring are chiefly diploids which show both paternal and maternal characters, indicating that they have been derived from  $1n$  egg cells, which are those resulting from the exceptional cases of second meiotic divisions. In a few cases the cross of a dyad by a normal gave a triploid which must have come from the union of a  $2n$  egg with a normal  $1n$  sperm. We have also had a dyad seedling come from pollinating a dyad plant with normal pollen. This we have interpreted as due to parthenogenesis of a  $2n$  egg.

Pollen of dyad plants has not been successfully used in crosses with normals, presumably because the tubes from grains with  $2n$  chromosomes burst in the styles of normal diploids (5). Such  $2n$  grains have approximately twice the volume of the  $1n$  grains found in normal diploids, and are easily distinguished from the latter. From the occasional admixture of  $1n$  grains among otherwise  $2n$  pollen, it can be concluded that a second division takes place in about 1 to 5 per cent of the pollen mother cells of dyad plants. Sometimes no  $1n$  pollen grains can be found in an anther, and rarely has the number reached more than 50 per cent.

### Discussion

As is known from the literature, various irregularities which occur during meiosis may result in the doubling of the entire chromosome complement. Only two such cases need be mentioned here: (1) BEADLE (2) has reported the failure of cytokinesis in maize after the first and second meiotic divisions or after only the second division. In such cases pollen grains with  $4n$  or with  $2n$  chromosomes are formed. (2) Another abnormality in sporogenesis, "the semi-hetero-

typic division," is described by ROSENBERG (7) in parthenogenetic *Euhieracium*. The first meiotic division is interrupted at various stages by a premature homoeotypic division. The entire spindle is surrounded by a nuclear membrane. A single "restitution nucleus" is formed which has the diploid number of split chromosomes. Since this homoeotypic division is the only one which is completed, dyad microspores with  $2n$  chromosomes are formed. Similar conditions have been found by other investigators. In the gene "dyad" which has been described here, it is the failure of the second meiotic division which leads to the formation of a diploid number of chromosomes in the gametes. The exact mechanism by which the gene suppresses the second division is not known, but the effect appears to be brought about through a premature cell wall formation which anticipates the second division, or, perhaps more likely, through a great extension of the telophase stage which in normal plants is of brief duration. Whatever may be the immediate effect of the gene by means of which it brings about dyad formation, its action is influenced by environmental factors, as shown by the exceptional cases of tetrad formation.

### Summary

1. Dyad formation in sporogenesis in *Datura*, owing to a recessive gene, was found among the  $F_2$  offspring from radium-treated pollen.
2. During megasporogenesis the first meiotic division is present, but the second meiotic division is regularly absent. As a result only two megaspores (dyads) are formed.
3. One of the megaspores disintegrates; the other undergoes three successive mitotic divisions. Its nucleus contains 12 pairs of chromosomes instead of 12 single chromosomes.
4. An embryo sac is formed containing eight nuclei, each with 24 chromosomes.
5. During microsporogenesis the same meiotic abnormality is found in the pollen mother cell. There is a failure of the second division, and after the first division a thick cell wall develops between the two nuclei, forming a dyad of microspores.
6. Each microspore, containing 12 pairs of chromosomes, develops into the male gametophyte.
7. A small percentage (about 1 to 5 per cent) of megasporocytes



and microsporocytes in dyad plants develops normally. Since both meiotic divisions are present, a "tetrad" of microspores instead of a "dyad" is formed. Each gamete that results from these exceptional cases contains 12 single chromosomes.

8. The formation of  $2n$  and  $3n$  normal offspring from the cross dyad  $\times$  normal confirms the occurrence of both  $1n$  and  $2n$  egg cells. Dyad  $2n$  offspring have come from parthenogenesis of a  $2n$  dyad egg.

9. The occurrence of large and normal sized pollen grains in dyad plants confirms the occurrence of both  $1n$  and  $2n$  sperms.

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# CULTURAL CHARACTERS AND PAIRING REACTIONS OF MONOSPOROUS MYCELIA AND DEVELOPMENT OF THE FRUIT BODY OF *PHOLIOTA* (*FLAMMULA*) *POLYCHROA*<sup>1</sup>

ALEXANDER H. SMITH AND HAROLD J. BRODIE

(WITH THIRTY FIGURES)

## Introduction

*Pholiota polychroa* (Berk.) Smith & Brodie was originally described from material collected at Waynesville, Ohio, but it has been found rather frequently in the region about the Great Lakes and along the eastern seacoast from Virginia northward, usually on partially decayed fallen trunks or branches of oak and maple. In the vicinity of Ann Arbor this fungus seems to grow most frequently upon oak, but may also be found on maple. In the northern peninsula of Michigan it has been found growing on hemlock as well as on oak. It would be desirable to make a comparative study of collections of *Pholiota polychroa* from both coniferous and deciduous woods, with a view to ascertaining whether or not two different strains exist, but the writers have not had the opportunity of doing this. All the material which served for the present study was collected from oak logs in the vicinity of Ann Arbor.

## Investigation

### GERMINATION OF BASIDIOSPORES

From a carpophore of *Pholiota polychroa* collected November, 1933, a spore deposit was obtained. The spores are rich dark brown in color and on the average measure  $6-7.5 \times 3-4 \mu$ . They germinated readily 12 hours after they had been sown on Kauffman's synthetic

<sup>1</sup> Papers from the Department of Botany and the Herbarium of the University of Michigan, no. 474.

As a result of their study of this fungus, as reported herewith, the writers propose to place it in the genus *Pholiota*.

agar (4), on malt extract agar,<sup>2</sup> or in an aqueous solution of malt extract.

✓ In order to study the behavior of nuclei during germination, spores were germinated in malt extract solution, were fixed and stained in the following manner. About 18 hours after the spores had been placed in the nutrient solution, the greater part of the liquid was decanted from the spores, which had settled to the bottom of the container. A quantity of weak Flemming's solution was then added to the residue and the spores allowed to stand for about one hour in the fixative. A thin layer of Szombathy's fixative was smeared over the surface of a glass slide and a drop of a 4 per cent solution of formalin was placed in the center of the slide. To this drop was added a drop of the solution containing the germinated spores. The slide was allowed to stand until the liquid had evaporated; it was then washed for 15 minutes in distilled water, bleached, and stained as desired. The use of Haidenchain's haematoxylin alone gave fairly good results. Particularly brilliant staining of the nuclei was obtained by the use of safranin.

The basidiospore possesses an apical pore through which the protoplasm of the spore emerges, forming a vesicle (figs. 1-4). That the germ pore is actually apical in position is demonstrated by the fact that in sections of the hymenium, spores still attached to the sterigmata of the basidia were seen to be provided with germ pores at their apical ends (fig. 20).

Two nuclei are usually present in the mature spore (figs. 1, 20). Upon germination, these nuclei migrate into the vesicle. Figure 2 shows one nucleus in the spore and one in the vesicle, while in figures 3 and 4 there are two nuclei in the vesicle.

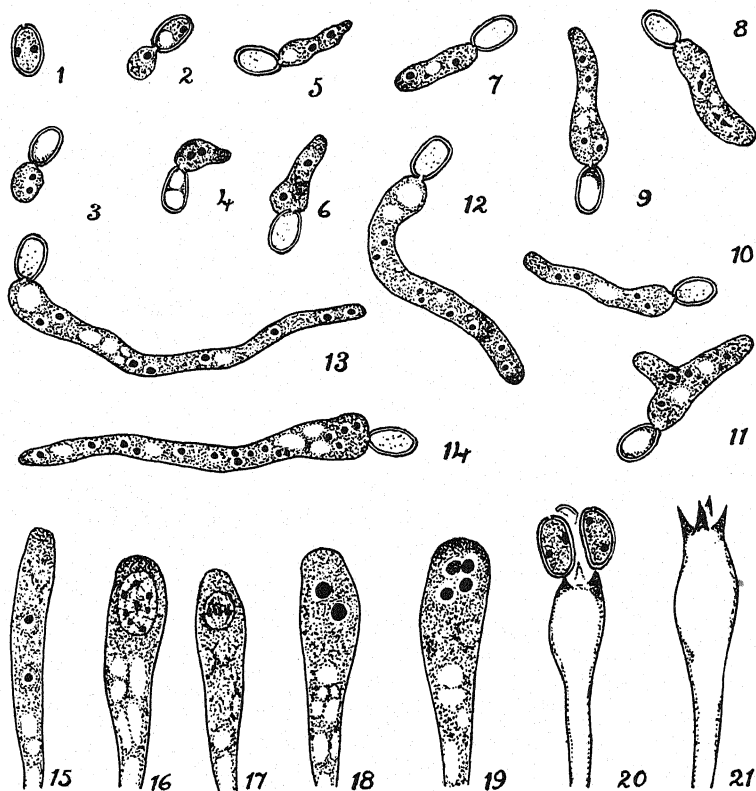
As the germ tube begins to develop from the vesicle (figs. 5, 6, 7), the two nuclei divide simultaneously (fig. 8), giving rise to four (figs. 9, 10, 11); and as development proceeds these give rise to eight, sixteen, and more nuclei (figs. 12, 13, 14). That the number of nuclei observed was either two, four, eight, or sixteen indicates that they divide simultaneously, a conclusion which is further upheld by their disposition in pairs (fig. 12). No attempt was made to follow the

<sup>2</sup> Prepared according to the following formula: agar 15 gm., malt extract (Merck and Co.) 15 gm., distilled water 1 liter.

multiplication of nuclei beyond the point where there were sixteen present. At this stage the young mycelium is still aseptate.

#### CULTURAL STUDIES AND ANALYSIS OF SEXUALITY

The mycelium which develops from a single basidiospore of *Pholiota polychroa* is haploid. It is composed of rather fine hyphae, the



FIGS. 1-21.—Figs. 1-14, stages in germination of basidiospores.  $\times 900$ . Figs. 15-21, nuclear history and basidiospore formation: fig. 15, primary nuclei before fusion; figs. 16, 17, fusion nuclei; fig. 18, two nuclei produced by first division of fusion nucleus; fig. 19, four nuclei in basidium prior to spore formation; fig. 20, mature basidium showing two spores only (note the two nuclei in each basidiospore); fig. 21, empty basidium showing no nuclei remaining after spores have been shed.  $\times 1130$ .

average width of which is about  $4\mu$ . The mycelium tends to lie flat on the agar substratum and does not present a fluffy appearance.

Oidia are produced in abundance on the haploid mycelium by the fragmentation of special spirally coiled hyphae. The oidia were found to germinate under suitable conditions.

Thirty single-spore mycelia were obtained from the spore deposit. They were grown on malt extract agar in tubes. Two months after they had been isolated, the mycelia were paired in all possible combinations on agar slants (435 pairings). Two weeks later these pairs were examined. In some of the tubes the two mycelia had intergrown without any apparent reaction having taken place between them. In other tubes the two mycelia had given rise to a diploid mycelium.

The diploid mycelium develops in the region of contact of the two haplophytes and then proceeds to grow over them. It differs from the haploid mycelium in producing a dense aerial growth. The development of diploid mycelium in a restricted area between the compatible haplophytes has been discussed by VANDENDRIES (8) in connection with his studies of *Trametes suaveolens*; and unpublished studies by Mrs. C. A. ARNOLD in this laboratory have demonstrated that a restricted diploidization also exists in certain species of *Collybia*. The difference in appearance between the haploid and diploid mycelium is illustrated by figure 22.

The appearance of the diploid mycelium developed between the two compatible haplophytes in culture tubes was so distinctive that macroscopic examination alone indicated which pairs of haplophytes were sexually compatible and which incompatible. The pairings of mycelia nos. 1 and 2 with each of the other 28 mycelia were examined also microscopically. In every instance clamp connections were found to be present on mycelia which had been marked as showing a positive reaction, and no clamp connections were found on mycelia which showed a negative reaction.

The behavior of the 30 single-spore mycelia was exceedingly regular. In none of the cultures examined microscopically were there any "false clamp connections" (9). In all the pairings, the appearance of diploid mycelium between any two haplophytes was always in accordance with the genetic formulae which could be ascribed to them from the results of their reactions with the other mycelia of the series. In none of the pairings was there any sign of the "barrage" phenomenon recently described by VANDENDRIES and BRODIE (9).

The results of the pairing experiment are incorporated in table I. In this table a plus sign indicates the presence of diploid mycelium; a minus sign, its absence.<sup>3</sup> It may be concluded that *P. polychroa* is heterothallic and tetrapolar.

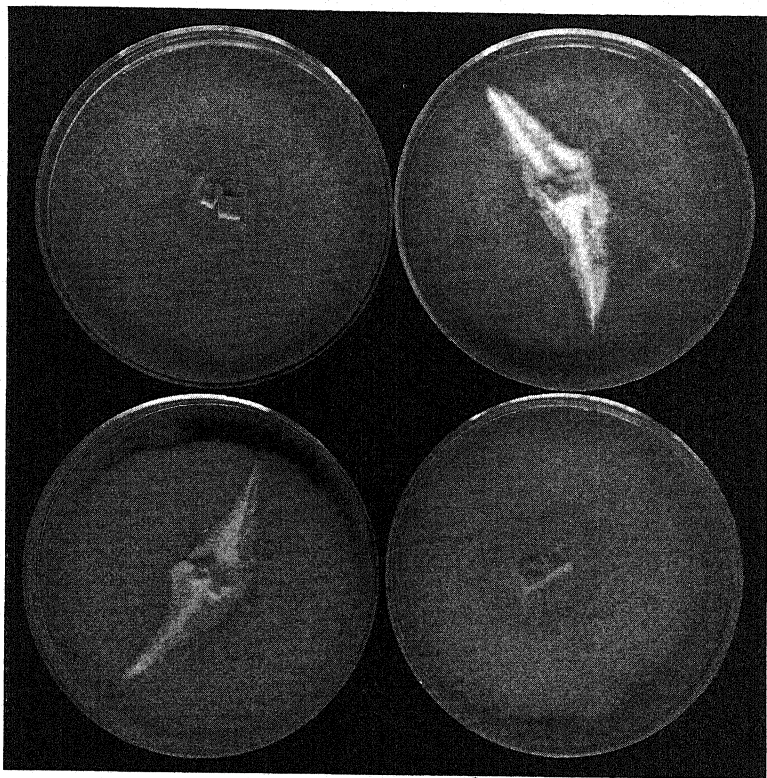


FIG. 22.—Haploid mycelia paired on agar plates showing localized development of diploid mycelium. Haploid mycelium grows compactly along surface of agar whereas diploid mycelium forms fluffy white aerial growth.  $\times 0.5$ .

Attempts were made to induce sporophore formation on diploid mycelium. Three flasks each of oatmeal, cornmeal, 2.5 per cent malt extract agar, and ETTER's medium (1) were prepared. In the last, finely ground sawdust (chiefly of oak and maple) was used. The

<sup>3</sup> Cultures 1, 2, 3, 4, 5, 6, 8, and 9 have been deposited in the Centraal Bureau voor Schimmelcultures at Baarn, Holland.

flasks were inoculated on December 2, 1931, and were placed in a darkened room maintained at a temperature of about 20° C.

The mycelia grew luxuriantly but no fruit bodies developed. The cultures eventually became dry. At the end of January, the mycelia

TABLE I

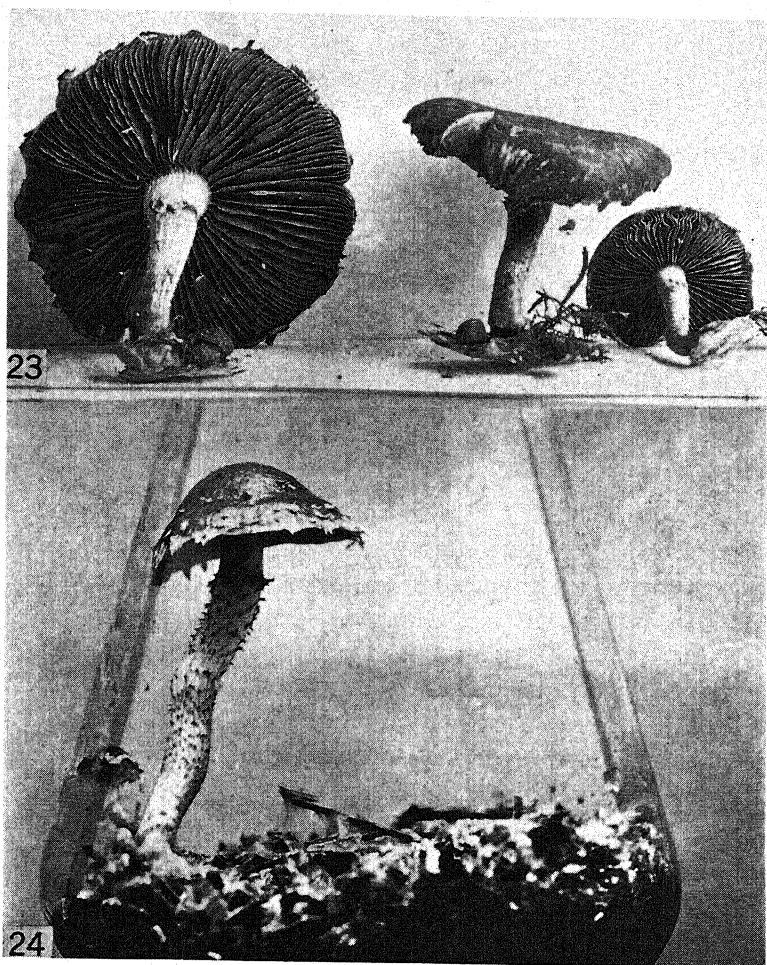
RESULTS OF PAIRING IN ALL POSSIBLE WAYS THIRTY SINGLE-SPORE MYCELIA OF  
PHOLIOTA POLYCHROA

	AB						ab						Ab						aB											
	8	9	15	21	23	29	30	1	2	10	11	12	16	18	24	27	5	6	17	19	22	26	3	4	7	13	14	20	25	28
AB	8	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	9	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	15	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	21	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	23	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	29	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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	10	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	11	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	12	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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	18	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	24	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	27	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
aB	17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
	19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
	22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
	26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-
	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-
aB	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-
	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-
	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-
	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-
	25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-
	28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-

on ETTER's medium were moistened with a sterile solution of 2.5 per cent malt extract and by the end of February a few malformed rudiments of fruit bodies were found in one culture. Again malt extract solution was added, but no further development of the rudiments took place.

On March 13 the three cultures on ETTER's medium were placed on a table near a window facing east, but not in direct sunlight, and

again moistened with malt extract solution. By April 1, button stages of sporophores had developed on the mycelium in one flask, and on April 8 a fruit body 4 cm. broad had matured. The sporo-



FIGS. 23, 24.—Fig. 23, fruit bodies collected and photographed by E. B. MAINS near Munising, Mich., 1932; fig. 24, fruit body which developed in culture on ETTER's medium, April 8, 1932.  $\times 1$ .

phore was in every respect typical (fig. 24), having all the characters ascribed to the species by KAUFFMAN (3).



The cultures were kept moist and eventually three crops of sporophores were obtained. The second developed about four weeks after the first and the last crop three weeks after the second. The spores from the fruit bodies produced last were only about 25 per cent germinable, although spores from the first crop of fruit bodies were nearly 100 per cent germinable. Only one of the three cultures on ETTER's medium produced any fruit bodies.

Experiments made later under more carefully regulated conditions failed to provide more sporophores, and it has not been found possible up to the present time to manipulate the conditions of culture so that the fruit bodies of *P. polychroa* can be produced when desired.

#### DEVELOPMENT OF FRUIT BODY

The material used for the developmental studies and the carpophore which provided the foregoing cultures were both obtained from the same locality. Material was killed and fixed in Allen's modification of Bouin's solution and was imbedded in paraffin in the usual manner.

**STEM PRIMORDIUM.**—The youngest fruit body sectioned was somewhat egg-shaped and its stem primordium was situated apically. Figure 25 represents a stage of development in which the stem fundament has just reached the upper surface of the basidiocarp primordium and begun the formation of the stipe. The writers believe, as SAWYER (7) and others have pointed out, that differentiation of the stem fundament actually begins near the base of the small, compact, subglobose mass of hyphae forming the fruit body primordium. At the stage of development shown in figure 25, the broad base is made up of compactly interwoven hyphae which measure 4–6  $\mu$  in diameter and which stain lightly but uniformly. Just above this area, in the oval part of the young fruit body, the hyphae stain more intensely and measure only 2–3  $\mu$  in diameter. The ground tissue located next to this dark area (which is the stem fundament) is also made up of narrow hyphae, but their arrangement is looser than that of the hyphae in the central layer.

**DIFFERENTIATION OF HYMENOPHORE AND PILEUS PRIMORDIA.**—The earliest stage found illustrating the development of the hymenophore primordium is shown in figure 26. Here a narrow band of

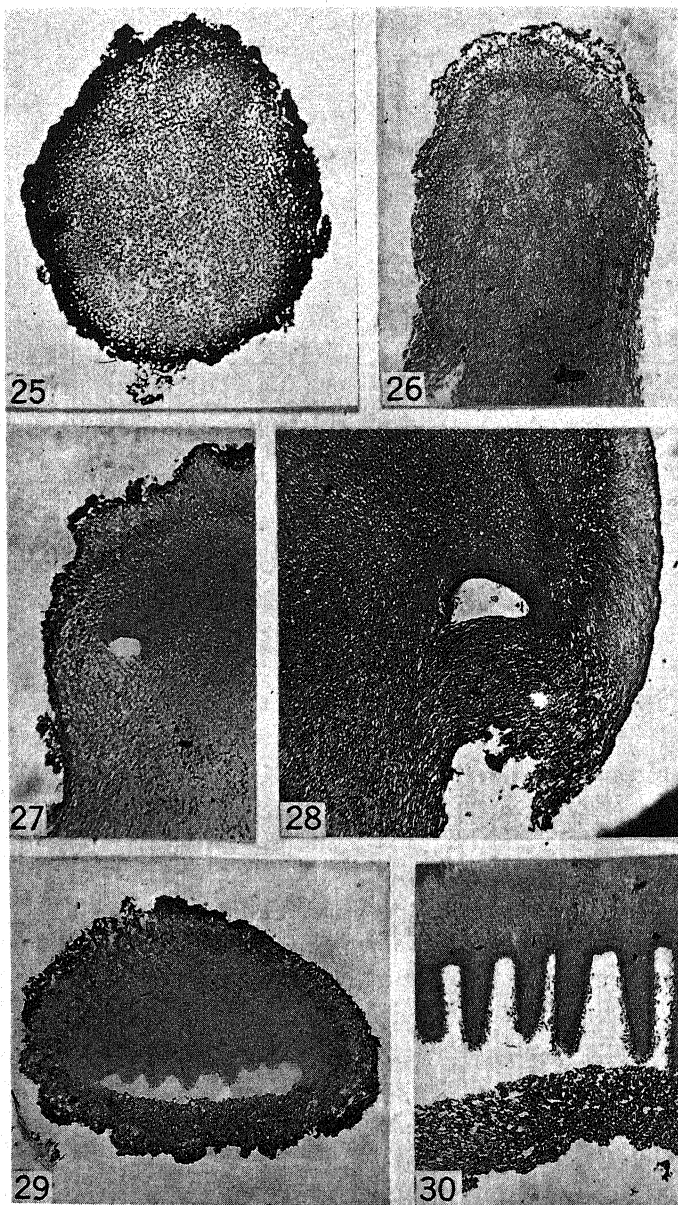
darkly staining hyphae can be seen, the ends of which point more or less toward the base of the basidiocarp. The tissue immediately beneath these hyphae stains lightly and is of very loose structure. At this stage there is no clearly defined palisade layer nor gill cavity.

A rather broad zone of narrow, deeply staining hyphae differentiates the pileus from the universal veil tissue, which stains lightly. The hymenophore and pileus primordia are separated by a narrow band of hyphae. It has been impossible to determine with certainty which of these two primordia is differentiated first. Apparently they originate at about the same time.

In figure 27 the blematogen layer can be recognized. This is very distinct in figure 28, which illustrates a young sporophore 3 mm. in width, because of the difference in the capacities of the two tissues for holding the stain. Eventually the greater part of the universal veil tissue becomes gelatinized, and as the cap expands, the tissue becomes thinner and more amorphous. In carpophores with well developed blematogenous tissue only the lower portion gelatinizes; and as the pileus expands, floccose patches are left scattered over the surface of the pileus, particularly on the margin. Both the partial and the universal veil contribute, however, to the formation of the floccose scales on the margin. In some fruit bodies only marginal scales are found, either because the entire blematogenous layer gelatinizes or because the small scales are removed mechanically during the expansion of the fruit body.

ORIGIN OF GILL CAVITY, LAMELLAE, AND PARTIAL VEIL.—As the hyphae grow downward to form the palisade layer (fig. 27), the ground tissue beneath becomes torn. At first the cavity thus produced is crossed by occasional hyphae; later it enlarges and is clearly defined (fig. 28). The palisade layer is rather uneven at first, but soon becomes very compact near the stipe.

The lamellae originate as obtuse folds near the stipe, and grow downward into the gill cavity as well as outward toward the margin of the pileus. The gill primordia can be distinguished (fig. 29) as areas of fine, darkly staining hyphae which push down on the palisade layer, eventually giving rise to definite folds which are the young gills. The growth of the gills continues and soon the narrow lamellae are easily discernible in the gill cavity (fig. 30). Growth



FIGS. 25-30.—Fig. 25, young basidiocarp showing stem primordium,  $\times 36$ ; fig. 26, same showing differentiation of pileus and hymenophore primordia,  $\times 34$ ; fig. 27, development of gill cavity and further differentiation of universal veil,  $\times 20$ ; fig. 28, gelatinization of universal veil (note that gelatinization progresses only a short distance beyond margin of pileus),  $\times 48$ ; fig. 29, development of gill primordia and formation of young gills,  $\times 50$ ; fig. 30, young gills and veil in partly expanded fruit body,  $\times 26$ .

may continue until the edges of the gills come into contact with or are even pushed into the veil tissue at the base of the cavity.

Both the partial and the universal veil are distinguishable at their points of conjunction with the pileus but become indistinguishable as they merge into the membranous veil. The gelatinization of the universal veil (fig. 28) progresses only slightly beyond the margin of the pileus. As the pileus expands and the stipe elongates, the veils are ruptured, leaving innate scales on the stipe and soft fibrillose or membranous patches scattered on the pileus, or appendiculate along the margin.

The cytology of basidiospore formation follows the usual course. Two primary nuclei (fig. 15) fuse and two divisions of the fusion nucleus follow (figs. 16-19). One nucleus migrates into each basidiospore and there divides so that the mature spore contains two sister nuclei (fig. 20). The writers could find no indication of any nuclei left behind in the basidium after the spores had formed and matured (figs. 20, 21) and are therefore certain that the binucleate condition of the mature basidiospore results from the division of a single nucleus.

The development of this fungus agrees in all essentials with the development of species of *Pholiota* studied by SAWYER (7). The development of the stem, pileus, and hymenophore are not of great importance because they are similar in most endogenously developing agarics except those of the *Amanita* type. In *Pholiota polychroa* the morphology and mode of development of the universal veil and the partial veil are practically identical with the development of these parts in *P. flammans* and *P. adiposa* (7). Inasmuch as little is known concerning the development of the species of *Flammula*, it is at present impossible to make a detailed comparison between the two genera *Flammula* and *Pholiota* from the standpoint of the development of the fruit body, or to attempt to establish generic limits on the basis of developmental studies. Undoubtedly such a study must be made before all the border-line species can be properly classified according to their natural relationships.

#### TAXONOMIC POSITION OF PHOLIOTA POLYCHROA

This fungus has at different times been referred to *Hypholoma*, *Pholiota*, and *Flammula*. PECK, who placed the species in the sub-

genus *Hypholoma* under *Agaricus* (6), later removed it to *Pholiota* (*P. ornella*), apparently after he had carefully studied the color of the spore print. The spores are of a rich dark brown color but not purple-brown as in *Hypholoma*. Whether the species under consideration more properly belongs in *Pholiota* or in *Flammula* is a matter difficult to decide, because the distinction between the two genera rests largely upon the character of the veil; the degree of its differentiation rather than its presence or absence has been considered of importance in the past.

Concerning the relationship of the two genera, FRIES (2) says of *Pholiota*, "sine distinctis limitibus transit in *Flammulas*"; and OVERHOLTS (5), "At some points the genus grades into *Flammula* due to the early disappearance of the partial veil or the annulus, and in *Flammula* the veil fragments may at times persist as an incomplete annulus." Such statements indicate that in placing collections in either genus one is often guided more by the opinions of previous workers than by the characters of the fungi in hand. The prominence of the well developed veil in the ontogeny of the fruit body of *Pholiota polychroa* indicates a closer relationship to certain species of *Pholiota* than to other species of *Flammula*.

Macroscopically *P. polychroa* is a typical border-line species. It is often found to possess the soft floccose scales on the pileus and along the margin and a definite subapical annular zone around the stipe. The stipe may also be covered with soft innate scales for some distance below the annular zone. These characters indicate a relationship to *Pholiota flammans* and *P. adiposa*.

Under relatively dry conditions, sporophores may be nearly destitute of a veil and the pileus may be entirely glabrous. Such specimens, which are frequently collected, no doubt account for the species being retained in *Flammula*. The fruit bodies shown in figure 23 illustrate this condition, but even here the annular zone is clearly visible.

From the preceding account it is apparent that there is not sufficient reason for keeping the fungus in the genus *Flammula*. The species shows a striking relationship to *Pholiota* in the manner of its development and in its stature, and it is distinguished from *Flammula* by possessing a veil which is well developed. The writers be-

lieve the fungus is more properly classified as a *Pholiota*, and therefore propose the combination *Pholiota polychroa* (Berk.) comb. nov.

#### SYNONYMS

- Agaricus polychrous* Berk.—Lond. Jour. Bot. 6:313. 1847.  
*Agaricus ornellus* Pk.—Ann. Rep. New York State Mus. 34:42. 1883.  
*Flammula polychroa* Sacc.—Syll. Fung. 5:824. 1887.  
*Pholiota appendiculata* Pk.—Bull. New York State Mus. 94:33. 1905.  
*Pholiota ornella* Pk.—Bull. New York State Mus. 122:151. 1908.  
*Gymnopilus polychrous* (Berk.) Murr.—North Amer. Flora 10: 204. 1917.

#### Summary

1. The basidiospores of *Pholiota polychroa* germinate readily on various media in 12–18 hours. A large germinal vesicle is formed first and the two nuclei present in the mature spore migrate into it before dividing. As the vesicle and germ tube develop, nuclear division takes place. In the early stages of the development of the haploid mycelium, the nuclei divide simultaneously and ceonocytic cells with two, four, eight, and sixteen nuclei are commonly found.

2. A pairing experiment using 30 monosporous mycelia showed this species to be heterothallic and tetrapolar. Diploidization takes place only along the line of contact between the two compatible haplophytes. The diploid mycelium grows slowly out over the haploid mycelium.

3. Typical sporophores producing germinable spores were obtained in pure culture, but it has not been possible as yet to manipulate the conditions of culture so that sporophores can be developed as desired.

4. It was found that the lamellae of the fruit body develop endogenously and that a pronounced gill cavity is formed. The manner of development and the type of veil produced indicate a close relationship to species of *Pholiota*, and the species is transferred from *Flammula* to *Pholiota*.

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# SALIENT LINES OF STRUCTURAL SPECIALIZATION IN THE WOOD RAYS OF DICOTYLEDONS

DAVID A. KRIBS\*

(WITH PLATE VIII AND ONE FIGURE)

## Introduction

In recent years numerous attempts have been made to establish correlations between anatomical characters of the stem and morphological characters of the flowers, fruits, and leaves in a supposedly natural classification of the dicotyledons. The results obtained thus far are more or less contradictory. High correlations frequently occur in groups of closely related species and genera, whereas serious discrepancies arise in the case of large families and orders. If serious confusion is to be avoided, wood technologists should devote more attention to the study of the main lines of structural specialization in the evolutionary development of the dicotyledons as a whole.

BAILEY and his co-workers (2, 3, 4, 6, 7, 8) have shown that the salient lines of structural specialization in the cambium and tracheary elements of dicotyledons are so distinct and so closely correlated that they may be studied effectively by statistical methods. Their investigations demonstrate that the fusiform initials of the cambium and their derivatives are reduced in length as specialization increases. As the vessels of the secondary xylem become more and more highly differentiated, their segments gradually lose their resemblance to tracheids, and tend to become progressively wider and shorter. At the same time the structure and arrangement of the bordered pits in their lateral walls tend to be considerably modified. Furthermore, the surrounding tracheary elements tend to shorten and to take on a more fiber-like structure, their pits becoming vestigial by the gradual disappearance of the bordering areas of the secondary walls.

The writer has endeavored to trace major lines of specialization in the wood rays and wood parenchyma of dicotyledons. The present paper deals with the wood rays; the distribution of wood parenchyma will be discussed in a subsequent paper.

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### Methods

Methods essentially similar to those of BAILEY and TUPPER (4) and FROST (6) were followed, that is, the statistical method was applied to the results of a detailed anatomical investigation of the woods in Professor BAILEY's collection. The collection is representative of the woods of the world and in 1929 it included 63 of the 76 orders and 137 of the 264 families listed by HUTCHINSON, approximately 800 genera and 2500 species being represented. Since that time numerous woods from various parts of the world have been added to the collection.

As FROST (6) has pointed out, "the statistical method is essentially a method for the study of the characteristics of a large group by taking individual samples, at random, from the group. Experience in agreement with theory has repeatedly shown that the accuracy of conclusions drawn from statistical results is proportional to the number of cases used in deriving the statistical constants. For this reason the number of individual woods measured and studied in these investigations is large in order to insure a fair representation of dicotyledonous woods in general."

The woods were divided into six groups upon the basis of the structure of their tracheary tissue, as follows: scalariform I, scalariform II, scalariform-porous, porous-oblique, porous-oblique and transverse, and porous-transverse. The ray types and wood parenchyma types were then studied in detail for each group separately. This procedure reveals certain diagnostic criteria which might otherwise be overlooked.

### Discussion

Hitherto wood rays have been placed in the following categories: uniseriate, compound, aggregate, multiseriate, heterogeneous, and homogeneous. According to the latest publication by the Committee on Nomenclature, International Association of Wood Anatomists (12), three types of wood rays are recognized: *aggregate ray* (a group of small, narrow, xylem rays appearing to the unaided eye or at low magnification as a single large ray); *homogeneous ray* (a xylem ray composed of radially elongated cells); *heterogeneous ray* (a xylem ray composed of cells of different morphological types, typically with

the cells of the multiseriate part radially elongated and those of the uniseriate parts vertically elongated or square).

One would infer, from these definitions of the terms *homogeneous* and *heterogeneous*, that the Committee has restricted the use of these terms exclusively to the multiseriate ray. Concomitant *uniseriate* rays evidently have not been taken into consideration. This is especially important, not only from the standpoint of formulating keys to the identification of woods, but also because of its great assistance in elucidating a phylogenetic sequence. It is suggested that any subsequent terminology should include the uniseriate ray in association with the multiseriate, thus eliminating the present ambiguous application of the terms.

In correlating wood rays with the various types of vessel elements, certain specific ray combinations were conspicuously brought to the writer's attention: (1) wood rays may be divided into six distinct types; (2) in two distinct ray types, the cells which constitute the uniseriate rays differ in size and form from the cells of the multiseriate portion of the multiseriate rays; (3) conversely, in two other distinct ray types, the cells which constitute the uniseriate rays are identical with those of the multiseriate rays. The rays of the stems containing the first two types are classified as heterogeneous and the rays of stems containing the second two types are classified as homogeneous. The question of terminology concerning the multiseriate ray in each type is left to the Committee on Nomenclature. *Heterocellular* is suggested for the ray in which the interior cells are radially elongated and the marginal cells vertically elongated or square; *homocellular*, for the ray in which the cells are all radially elongated.

### Ray classification

#### I. HETEROGENEOUS TYPE I (fig. 1)

1. Uniseriate rays usually high, numerous, and composed of very large, vertically elongated cells which are unlike the cells of the multiseriate part of the multiseriate rays.
2. Multiseriate rays usually with parallel sides and with very large, vertically elongated, uniseriate wings (long wings) which are composed of cells identical with those of the uni-

seriate rays. The cells of the multiseriate portion of the ray are oval, radially elongated, or vertically elongated.

## II. HETEROGENEOUS TYPE II (fig. 3)

Uniseriate rays usually lower and composed of cells which are unlike those of the multiseriate part of the multiseriate rays.

- A: 1. Uniseriate rays composed of rectangular, vertically elongated cells only.
2. Multiseriate rays with sides parallel or fusiform, the cells of the multiseriate portion being round to oval, radially elongated, and with uniseriate tips of large, vertically elongated cells (short wings); or with large, vertically elongated marginals, one cell high.
- B: 1. Uniseriate rays of two types; some of the uniseriates are composed of rectangular, vertically elongated cells; some are composed of cells which are nearly identical with those of the multiseriate part of the multiseriate rays.
2. Multiseriate rays with sides parallel or fusiform, the cells of the multiseriate portion being round to oval, radially elongated, and with medium to small, vertically elongated marginals, usually single, occasionally two cells high. If the uniseriate tips are longer, the cells are modified; that is, they are mostly square.

## III. HOMOGENEOUS TYPE I (fig. 4)

1. Uniseriate rays rather low, numerous to scarce, and composed of cells which are identical with those of the multiseriate rays.
2. Multiseriate rays mostly fusiform, the cells of the multiseriate portion being round to oval, radially elongated, and with long to short, uniseriate tips composed of cells identical with those of the multiseriate portion of the ray. Occasionally a multiseriate ray possesses very small square marginals, one cell high, which occur sporadically.

## IV. HOMOGENEOUS TYPE II (fig. 2)

1. Uniseriate rays usually scarce to absent. When present, low, and composed of cells identical with those of the multiseriate rays.

2. Multiseriate rays fusiform, and composed entirely of small, round, radially elongated cells; uniseriate tips absent or extremely short.

V. UNISERIAL RAYS ONLY: HETEROGENEOUS TYPE III (fig. 5)

VI. UNISERIAL RAYS ONLY: HOMOGENEOUS TYPE III (fig. 6)

Table I shows the percentage of ray types in each vessel type. Scalariform I represents the most primitive type of vessel element; porous-transverse, the most highly specialized type. In stems which

TABLE I  
PERCENTAGE OF RAY TYPES IN EACH VESSEL TYPE

TYPE OF VESSEL ELEMENT	NUMBER OF GENERA	RAY TYPES					
		MULTISERIAL AND UNISERIAL				UNISERIAL ONLY	
		HETEROGENEOUS		HOMOGENEOUS		HETERO- GENEOUS TYPE III	HOMO- GENEOUS TYPE III
		TYPE I	TYPE II	TYPE I	TYPE II		
Scalariform I.....	63	79.36	15.87	.....	.....	4.77	.....
Scalariform II.....	32	53.12	21.87	12.50	3.13	9.38	.....
Scalariform-porous.....	67	47.77	35.81	5.98	4.48	2.98	2.98
Porous-oblique.....	220	44.09	43.18	5.45	3.56	2.36	1.36
Porous-oblique and trans- verse.....	148	9.45	33.11	34.45	9.49	2.02	11.48
Porous-transverse.....	220	.....	19.09	27.28	44.10	1.81	7.72

possess both multiseriate and uniseriate rays, there is a high correlation between ray type and vessel element type; that is, the heterogeneous type I rays predominate in the stems with the scalariform type of vessel element, whereas the heterogeneous type II rays are very conspicuous in the scalariform-porous, porous-oblique, and porous-oblique and transverse groups. On the other hand, the homogeneous type I rays are dominant in the porous-oblique and transverse, and the porous-transverse groups; while the homogeneous type II rays are dominant in the porous-transverse group.

Reading across the table in each vessel element group, the heterogeneous type I rays constitute 79 per cent of the rays in the scalariform I group and 53 per cent in the scalariform II group. In the

scalariform-porous group, nearly 48 per cent are heterogeneous type I rays and nearly 36 per cent heterogeneous type II rays. In the porous-oblique group, the percentages of heterogeneous type I and type II are equal, being 87 per cent of the total number of ray types. In the porous-oblique and transverse group, there is an equal percentage of heterogeneous type II and homogeneous type I rays, or over 67 per cent of the total number of ray types; while in the porous-transverse group more than 71 per cent of the ray types are homogeneous, 44 per cent being homogeneous type II.

Table I shows rather conclusively that the heterogeneous type I rays, the multiseriates with their greatly elongate uniseriate wings, together with the high uniseriates composed of greatly elongate cells, are indeed the primitive type. As specialization continues, the multiseriates become more fusiform in outline, the wings become shorter, and the cells of the uniseriates become smaller, as illustrated by the heterogeneous type II rays. The next step in the evolutionary sequence involves a change from the heterogeneous to the homogeneous condition in which the cells of the uniseriates are identical with those of the multiseriates. In addition, the uniseriates are becoming shorter and less numerous and the multiseriates are mostly fusiform and homogeneous, as illustrated by the homogeneous type I rays. Finally, the most highly specialized condition is represented by the homogeneous type II rays in which the uniseriates are usually scarce or absent, the multiseriates being fusiform and homogeneous. Figure 7 illustrates diagrammatically the salient lines of structural specialization in the wood rays of dicotyledons.

Table II shows the total percentage of ray types in each vessel type. This table also indicates that the heterogeneous type rays are more primitive than the homogeneous ones. In the scalariform I vessel element group, the rays are 100 per cent heterogeneous; conversely, in the porous-transverse vessel element group, the rays are nearly 80 per cent homogeneous.

Tables I and II both indicate that during the process of evolutionary development the primitive type of ray is retained for long periods of time. This is especially true of some of the tropical plants in comparison with those of more temperate regions.

Table III shows a high correlation between ray type and vessel

element length. It indicates that as woody dicotyledonous stems become more highly specialized, the trend is from the heterogeneous type I ray to the homogeneous type II ray, and that heterogeneous rays in general are more primitive than homogeneous ones.

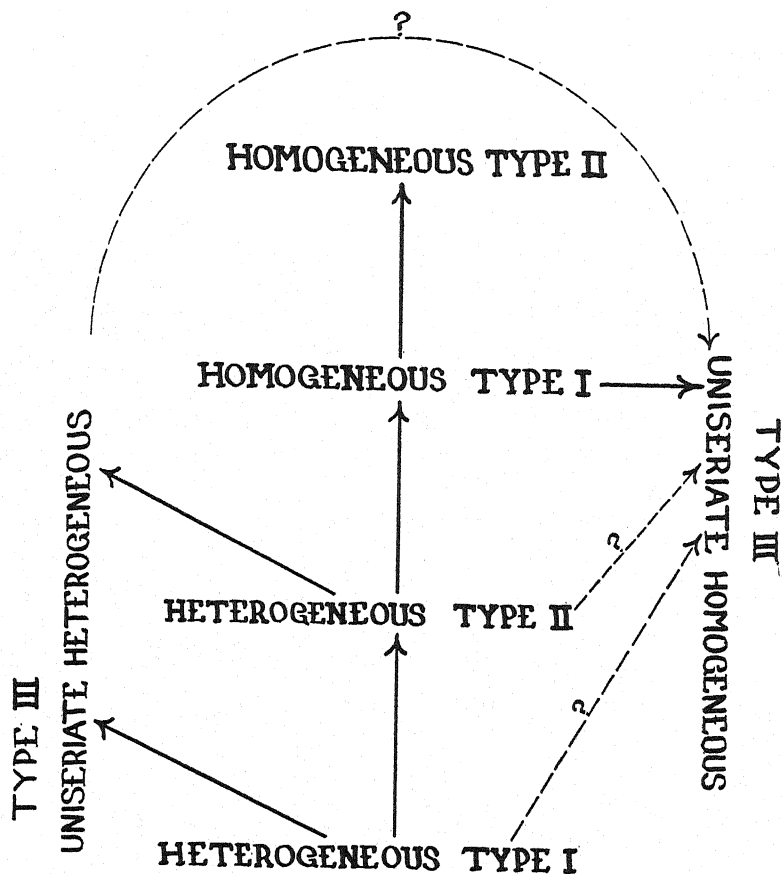


FIG. 7.—Diagrammatic illustration of salient lines of structural specialization in the wood rays of dicotyledons.

Taking into consideration the stems which possess uniseriate rays only, table I shows that there is no correlation between vessel element type and ray type, with the exception that the homogeneous uniseriate ray is more highly specialized than is the heterogeneous uniseriate.

The uniseriate ray occurs as an offshoot in practically every type of woody dicotyledonous stem, indicating that it is a specialized structure due to the elimination of multiserial rays. BAILEY (1) has

TABLE II  
TOTAL PERCENTAGE OF RAY TYPES IN EACH VESSEL TYPE

TYPE OF VESSEL ELEMENT	PERCENTAGE RAYS	
	HETERO-GENEUS	HOMO-GENEUS
Scalariform I.....	100.00	.....
Scalariform II.....	84.37	15.63
Scalariform-porous.....	86.56	13.44
Porous-oblique.....	89.63	10.37
Porous-oblique and transverse.....	44.58	55.42
Porous-transverse.....	20.90	79.10

TABLE III  
AVERAGE LENGTH OF VESSEL ELEMENT IN  
MM. FOR EACH RAY TYPE\*

TYPE OF RAY	NUMBER OF GENERA	AVERAGE ELEMENT LENGTH (MM.)
Heterogeneous type I.....	210	0.81
Heterogeneous type II.....	227	0.58
Homogeneous type I.....	131	0.52
Homogeneous type II.....	123	0.35
Uniseriate heterogeneous type III.....	18	0.64
Uniseriate homogeneous type III.....	41	0.38

\* Vessel element length indicates total element length, tip to tip, from macerated material.

shown this to be true for *Castanea* and *Alnus*, and HOLDEN (10, 11) reached the same conclusions concerning the rays of *Salicales* and of *Aesculus* in the *Sapindales*. Furthermore, GROOM (9) has suggested that the small rays of *Quercus* may have originated from the disintegration of primitive wide multiserial rays. The multiserial rays of oak, however, are not primitive but specialized structures.

CHATTAWAY (5), in her study of the ontogenetic development of

rays in the Sterculiaceae, also points out that uniseriate rays are derived by the gradual splitting of the multiseriate ray. She states also: "It appears that abundance of sheath-cells and the method of enlarging the rays at the expense of the fusiform initials is a primitive feature, and that the small-rayed species are on the whole more advanced than the large-rayed ones." A lack of material prevented a detailed study of all the genera of the Sterculiaceae; those examined, however, showed no correlation between the presence of sheath cells, size of ray, and ray type. Although some of the large-rayed species in which sheath cells are well developed possess the transitional heterogeneous type II rays, other large-rayed species in which sheath cells are only partially developed possess the same ray type. This is also true of some of the small-rayed species in the same category. The same results were obtained for the woods of group B, rays normally without sheath cells. The presence of sheath cells seems to indicate a distinct line of specialization restricted to the subfamily Sterculieae. This also seems to be true for tile cells in the rays of certain of the Malvales.

### Summary

1. Rays of woody dicotyledons may be classified into six salient types: heterogeneous type I; heterogeneous type II; homogeneous type I; homogeneous type II; uniseriate, heterogeneous type III; uniseriate, homogeneous type III.
2. There is a high correlation between vessel type and ray type, the evolutionary sequence being from the heterogeneous type I, the most primitive, through the transitional heterogeneous type II and homogeneous type I, to the highly specialized homogeneous type II rays.
3. The uniseriate, homogeneous type III rays are more highly specialized than the uniseriate, heterogeneous type III rays.
4. The uniseriate ray is a highly specialized structure owing to the elimination of multiseriate rays.
5. Specialization of rays is dependent upon the degree of heterogeneity rather than upon the size of the rays.
6. The so-called compound ray is merely an unusually wide multiseriate ray. Such rays are of common occurrence in both primitive



and specialized types of dicotyledons, and may be of either the heterogeneous or the homogeneous type.

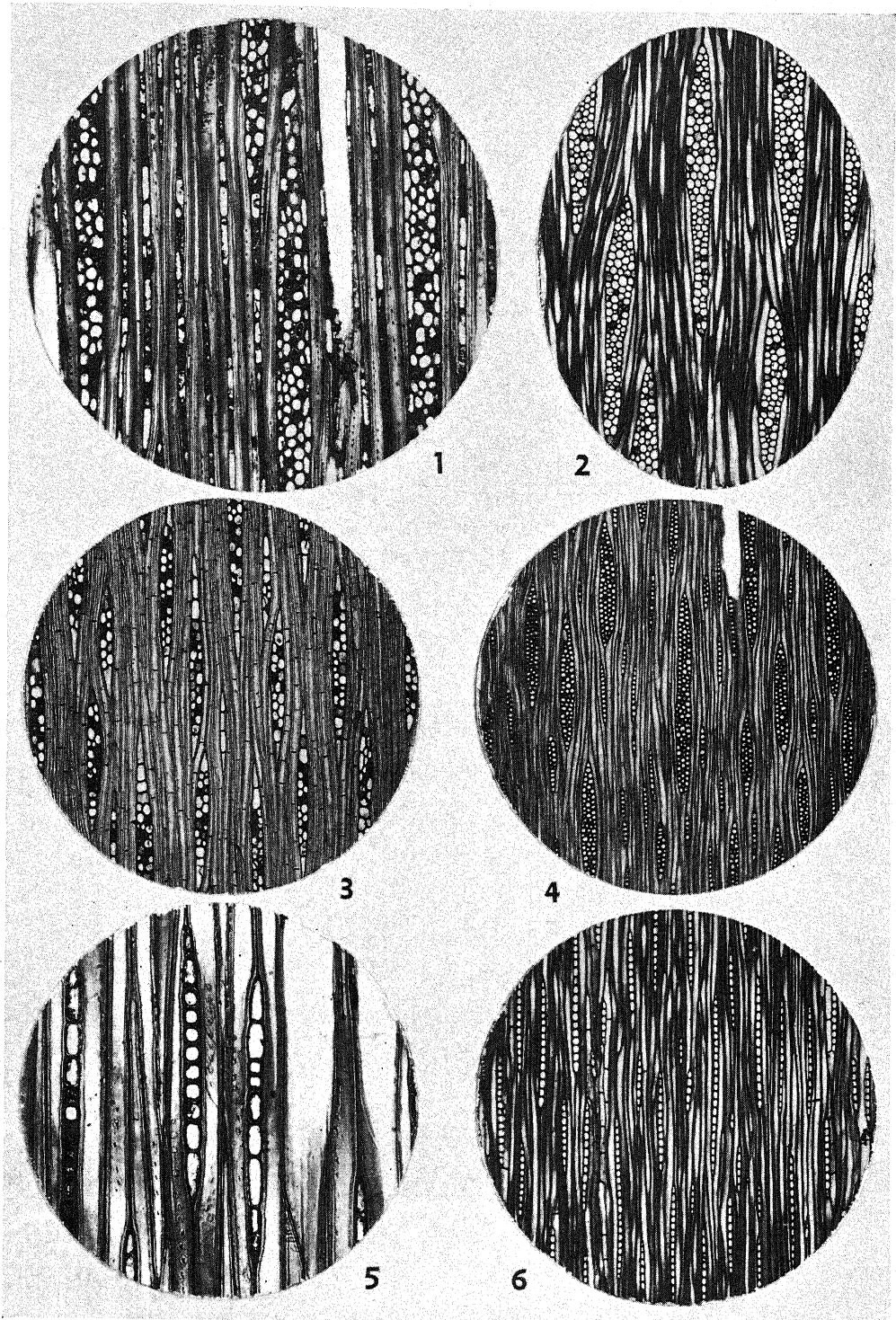
7. The so-called aggregate ray is a specialization which occurs sporadically. It is an offshoot from the main line of structural specialization in rays.

The writer expresses his thanks to the National Research Council for the grant which made this investigation possible, and to Professor I. W. BAILEY for his kind suggestions and advice during the progress of the investigation.

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# SOMATIC CHROMOSOME NUMBERS IN THE GENUS *SEDUM*

J. T. BALDWIN, JR.

(WITH FOURTEEN FIGURES)

## Introduction

This note is written as a preliminary report on a genetic and cytological study of the genus *Sedum*. The results ought to be of value in clearing up some of the problems of classification and of evolution within the group. Such application of the results from cytogenetic investigations is in accord with the more recent developments in taxonomic work.

No reports of genetic studies on any of the Crassulaceae and no references to extensive cytological investigations of this family have been encountered. ROCÉN (5) states that determinations of embryological relationships in the Crassulaceae are relatively few in comparison with those made for several other families of the order Rosales. The standard lists of chromosome numbers in plants contain few chromosome counts for this family; GAISER (2) records only the following:

	n	2n
<i>Bryophyllum calycinum</i> Salisb. . . . .	40	(38?) TAYLOR (1926)
<i>Penthorum sedoides</i> L. . . . .	8	ROCÉN (1928)

*Sedum* appears to be a genus suitable for cytogenetic investigation. Most species of the genus grow well from seed and may be transplanted without their growth being seriously checked. Most of the species are easy to propagate vegetatively; by this means plants of particular interest may be preserved. Annuals, biennials, and perennials exist; monoecious and dioecious species are known. The majority of the cultivated forms are hardy in our climate. Natural species hybrids, although rare, occur. A wide range of form, size, and color, both flower and foliage, is available for genetic analysis. The chromosome number, although large, has been found to vary

from species to species, and in one case within a species. The chromosomes of a complement, although the chromosomes are small, have been observed in root tip metaphases to be of a range of sizes. In many cases chromosome constrictions have easily been detected. These, with still other characteristics, would seem to recommend *Sedum* as favorable material for study.

According to HUBER (3), *Sedum* is the most widely distributed genus of the Crassulaceae; to this family BAILEY (1) assigns twenty genera. PRAEGER (4) estimates the genus to include about 500 known species, which are found in varying abundance throughout the Northern Hemisphere and below the equator in the Andes of Peru and Bolivia. The main habitat is central Asia, especially the mountains of West China. Ten generic subdivisions, established for the most part on general growth form, are recognized by PRAEGER (4). HUBER (3) observes that the geographic distribution of the genus coincides in the main with its morphological and systematic arrangement. To ascertain the relationships existing between the sections of the genus and their chromosome numbers, therefore, will be of interest from the viewpoint of the evolution of the various groups. In other plant genera a knowledge of chromosome numbers and of chromosome morphology has been helpful in determining species and in discovering interspecific relations. These two criteria of taxonomic position, applied on the basis of the systematic work already done on the groups, should be of much value in so polymorphic a genus as *Sedum*.

Taxonomically *Sedum* is not a particularly difficult genus. Some of its species are variable but the greater part of them are stable and distinct. In so far as its cultivated forms are concerned, however, *Sedum* is in a much confused state. In large measure this confusion is caused by the ease with which the plants propagate themselves asexually, thereby frequently spreading to areas supposedly prohibited to them, and by the dispersal, through growers, of all plants from a given plot as belonging to the form to which the plot had been assigned. This and the occurrence of names which have been given to no described species contribute much to the incorrect identification that is prevalent.

### Material and methods

Root tips were obtained from the adventitious roots of plants growing in the garden or of plants placed over water in the laboratory. They were fixed in Nawaschin's solution, cleared in xylene and imbedded in paraffin, sectioned at a thickness of  $7\ \mu$ , and stained according to Newton's gentian violet method. Fixation was usually good.

All drawings were made at table level with the aid of a camera lucida and at a magnification of  $\times 4900$ . A Zeiss microscope, equipped with a  $\times 30$  compensating ocular and a  $\times 90$  fluorite objective, n.a. 1.25, with yellow-green filters, was used.

### Results

The somatic chromosome numbers of twelve *Sedum* species, distributed among four sections of the genus, have been determined. Specific identifications assigned by growers have been checked with the descriptions found in PRAEGER (4) and HUBER (3). A form of *S. hispanicum* L. was obtained from the Valley View Greenhouses, Charlottesville, Virginia, as *S. glaucum*. The latter is a synonym for the former (Index Kewensis). Plants collected from the wild or from private gardens have likewise been identified except in two instances. One of the unidentified forms resembles *S. purpureum* Link; the other resembles *S. telephium* L. PRAEGER (4) places *S. purpureum* as a subspecies of *S. telephium*. The species investigated, the sections to which they belong, their somatic or  $2n$  chromosome numbers, and the sources from which the plants were obtained are given in table I.

The chromosomes in all the root tip metaphases examined were usually well spaced and found in one plane. As a result the counting was not difficult, except in some of the higher numbered forms in which it was not always easy to distinguish constrictions from actual separations.

### Discussion

The chromosome numbers of the species so far investigated form an irregular series apparently founded upon no common basic number. A regular series, however, is shown by the forms in one of the generic subdivisions investigated.

Section *Telephium* is composed of a group of about 25 hardy

perennial species which range across Eurasia from England to Japan. They are most abundant in the East. One species, however, *S. telephioides*, occurs in and is restricted to North America. The four forms of this group now investigated exhibit a chromosome

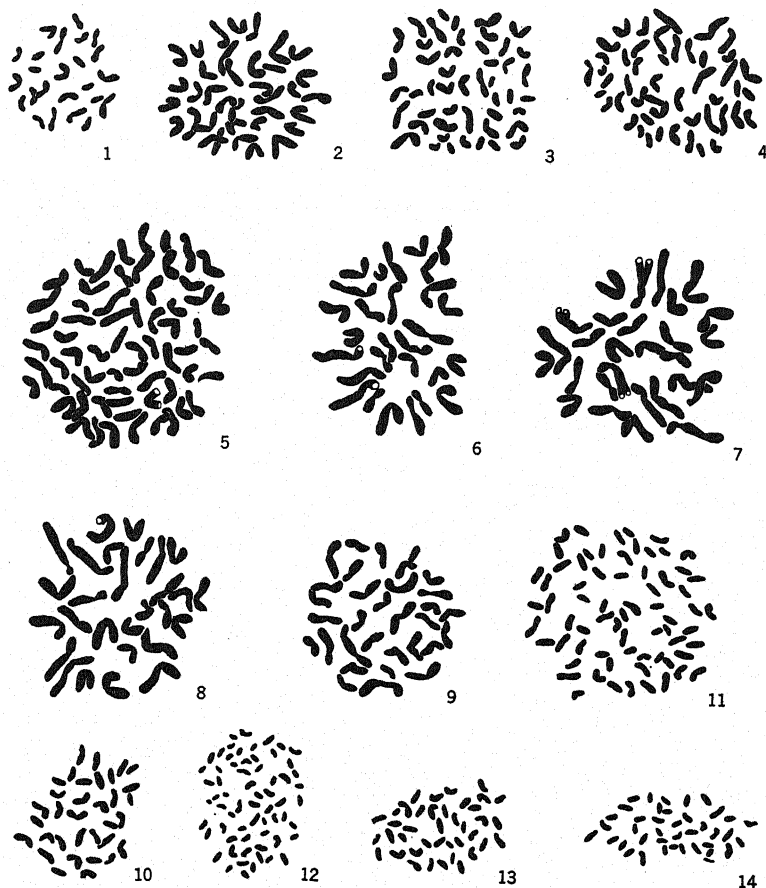
TABLE I

SECTION	SPECIES	2N CHROMO- SOME NUMBER	LOCALITY
Telephium S. F. Gray	<i>S. telephioides</i> Michx.	24	Wild, Shepherdstown, W. Va.
	<i>S. telephioides</i> Michx.	24	Ridgeville Nurseries Inc., Mount Airy, Md.
	<i>Sedum</i> sp. (resembles <i>S. pur- pureum</i> Link)	36	Cultivated, The Plains, Va.
	<i>Sedum</i> sp. (resembles <i>S. tele- phium</i> L.)	48	Cultivated, Charlottes- ville, Va.
	<i>S. spectabile</i> Boreau	48	E. B. Drake Alpine Gar- dens, Lakebay, Wash.
Aizoon Koch	<i>S. kamtschaticum</i> Fisch and Meyer	64	Valley View Greenhouses, Charlottesville, Va.
Seda Genuina Koch	<i>S. divergens</i> (?) S. Watson	28	" "
	<i>S. spurium</i> M. B.	28	" "
	<i>S. stoloniferum</i> S. T. Gmelin	28	" "
	<i>S. ternatum</i> Michx.	32	Wild, Boyce, Va.
	<i>S. reflexum</i> L.	34	Ridgeville Nurseries Inc., Mount Airy, Md.
	<i>S. reflexum</i> L.	68	Nursery near Washington, D.C.
	<i>S. album</i> L. <i>S. album</i> L.	64 64	" " Valley View Greenhouses, Charlottesville, Va.
Epeteium Boissier	<i>S. hispanicum</i> L.	40	E. B. Drake Alpine Gar- dens, Lakebay, Wash.
	<i>S. hispanicum</i> L.	40	Valley View Greenhouses, Charlottesville, Va.

series with a basic number of 12. *S. telephioides* has been found to have as a 2n number 24 chromosomes (fig. 1); *Sedum* sp. from The Plains, Virginia (possibly cultivated from the wild), has 36 (fig. 2); *Sedum* sp. from Charlottesville, Virginia (possibly from the wild), has 48 (fig. 3); and *S. spectabile* has 48 (fig. 4). Thus a series of 24-36-48 is present.

Only one chromosome number, that of *S. kamtschaticum* with 64

chromosomes (fig. 5), has been determined for section Aizoon. This section includes only a few species, which are exclusively limited to eastern Asia, and which give a fairly uniform impression.



FIGS. 1-14.—Metaphase plates from root tips of *Sedum*: fig. 1, *S. telephioides* with 24 chromosomes; fig. 2, *Sedum* sp., 36; fig. 3, *Sedum* sp., 48; fig. 4, *S. spectabile*, 48; fig. 5, *S. kamtschaticum*, 64; fig. 6, *S. divergens*?, 28; fig. 7, *S. spurium*, 28; fig. 8, *S. stoloniferum*, 28; fig. 9, *S. ternatum*, 32; fig. 10, *S. reflexum*, 34; fig. 11, *S. reflexum*, 68; fig. 12, *S. album*, 64; fig. 13, *S. hispanicum*, 40; fig. 14, *S. hispanicum*, 40.  $\times 2450$ .

Section Seda Genuina lacks a natural relationship, for in it are associated a number of forms that fit into none of the other groups. It has a wider distribution range and includes more species than any

other section of the genus. Six species of this section have been investigated. They are *S. divergens?* with 28 somatic chromosomes (fig. 6); *S. spurium*, 28 (fig. 7); *S. stoloniferum*, 28 (fig. 8); *S. ternatum*, 32 (fig. 9); *S. reflexum*, 34 (fig. 10) and 68 (fig. 11); and *S. album*, 64 (fig. 12). The 68-chromosome-numbered form of *S. reflexum* is more branched and less compact than the 34-chromosome-numbered form of the species. No progressive series of chromosome numbers can at present be arranged for the group as a whole.

Section Epeteium, made up of annual, rarely biennial, forms, is widely distributed. Only two forms of this section have been examined. They belong to *S. hispanicum*, and each has 40 somatic chromosomes (figs. 13, 14).

The chromosomes of none of the other six sections of the genus have yet been investigated. With the exception of section Rhodioli, which is scattered over most of the mountains of the north temperate zone, these other sections are small and restricted in their distribution.

Because of insufficient data, no comparison with work on other genera and no discussion of chromosome morphology will at present be attempted. Investigations now in progress will make such a treatment possible and will be reported in a later paper.

### Summary

1. Little cytogenetic research has been done on the Crassulaceae. The genus *Sedum* is good material for study.
2. The somatic chromosome numbers of twelve *Sedum* species, including fourteen forms distributed among four generic sections, are reported.
3. A chromosome series of 24–36–48, with a basic number of 12, is exhibited by the forms reported in section Telephium S. F. Gray.

The writer wishes to express his appreciation to Dr. ORLAND E. WHITE for his valuable criticisms during the course of this study and for making available the material with which the investigation is concerned.



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## CHROMOSOME NUMBERS OF SOME OF THE CACTACEAE

PALMER STOCKWELL

(WITH SEVENTEEN FIGURES)

Continuing his interest in the cacti (8), the writer has begun a cytological investigation of them, believing that studies of the chromosome numbers of this group might aid in the solution of its taxonomic problems. This paper is a report of preliminary findings in this direction.

Root tips of available species were fixed in a chromo-acetic-formalin mixture used in this laboratory. This formula is designated as CL<sub>9</sub> and is as follows:

SOLUTION A, 2 PARTS	SOLUTION B, 1 PART
100 cc. distilled water	0.5 gm. saponin
10 cc. glacial acetic acid	100 cc. 16% formalin solution
1 gm. chromic acid	
1 gm. potassium dichromate	
1 gm. urea	

(Mix solutions A and B immediately before use.)

The slides were stained with gentian violet, safranin, and orange G as described in a previous paper (7). With this procedure no particular difficulty was encountered in counting the chromosomes.

Eight species or forms of *Opuntia*, one of the more primitive groups of the cacti, were examined. These plants of the flat-jointed, prickly pear type had been transplanted from their natural habitats and kept in cultivation for different periods of time varying from a few months to several years. Of the tribe Cereae, a highly evolved group, nine species were investigated, most of them being greenhouse seedlings. They included specimens of the giant cactus of the southwestern United States (*Carnegiea*); a giant cactus of northern Mexico (*Lophocereus*); one of the Christmas cacti, an epiphytic form from Brazil (*Schlumbergeria*); one of the porcupine cacti of the California

desert (*Echinocereus*); and several pincushion cacti from California, Arizona, and Texas (*Neomammillaria*). The specimens of *O. polyacantha* were collected by Dr. GÖTE TURESSON of Lund, Sweden, during a visit to this country last year. A number of the other plants were provided by Professor JAMES I. MCMURPHY of Stanford University.

The known somatic chromosome numbers in the Cactaceae are recorded in table I. Those counted in this study are numbered 1 to 17 in the table and are also illustrated in figures 1-17. The species are arranged after the system of BRITTON and ROSE (1). The chromosomes of the species investigated are rather small and polyploidy seems to be a common phenomenon, with 11 as the most frequent basic chromosome number. The species within the tribe Cereeae are morphologically more closely related to one another than to any species of the tribe Opuntieae, and *vice versa*. This relationship is also indicated in the chromosome plates. In general the Cereeae have larger chromosomes and a smaller number of them. Furthermore, the basic chromosome number appears to be more variable in the tribe Cereeae than in Opuntieae.

In Cereeae the diploid number 24 was reported by SUGIURA (9). In the nine species of this tribe examined in the present investigation, 44, 22, or 18 chromosomes were found (nos. 9-17, table I).

In the tribe Opuntieae, JOHANSEN (5) has reported a diploid number of 22. In the eight species of this tribe here examined the numbers 66, 44, or 22 have been observed (nos. 1-8, table I).

So far as we know, therefore, the basic numbers of the tribe Cereeae are 9, 11, and 12 while the basic number 11 is the only one reported for the tribe Opuntieae. Large satellites were found in representatives of both tribes when the chromosome number was low, but they have not been observed in those having high chromosome numbers. The satellites of *Lophocereus schottii* are so large that one may suspect that they are constricted ends of the chromosomes themselves.

Generalizations based on the scanty data at hand are hazardous. Some interesting conjectures, however, may be made from a projection of the results obtained to date. The opuntias are the most northern of the cacti and they have the higher chromosome num-

bers. In the species *Opuntia polyacantha*, chromosomes have been counted in specimens from Colorado, Saskatchewan, southern Alberta, and Peace River Crossing in northern Alberta near the station

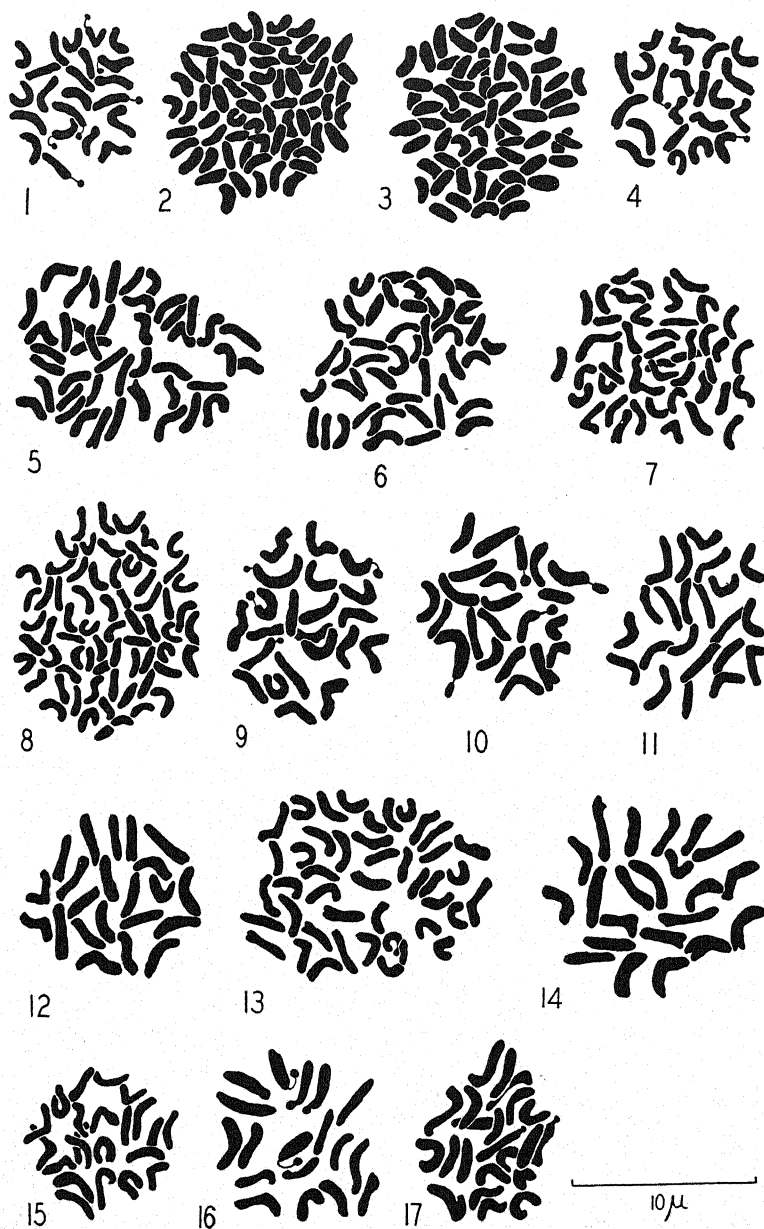
TABLE I  
KNOWN SOMATIC CHROMOSOME NUMBERS IN CACTACEAE

FIGURE NO.	NAME	APPROXIMATE DISTRIBUTION	2n
Tribe Opuntieae			
1	<i>Opuntia santa-rita</i> (G. & H.) Rose	Southern Arizona and northern Mexico	22
2	<i>O. phaeacantha</i> Engelm.	Texas to California and northern Mexico	± 66
3	<i>O. discata</i> Griffiths	Southern Arizona	± 66
4	<i>O. chlorotica</i> Engelm.	New Mexico to California	22
5	<i>O. polyacantha</i> Haw.—Colorado Springs	New Mexico to California and north to Canada	44
6	<i>O. polyacantha</i> Haw.—Saskatoon, Saskatchewan	New Mexico to California and north to Canada	44
7	<i>O. polyacantha</i> Haw.—Southern Alberta	New Mexico to California and north to Canada	44
8	<i>O. polyacantha</i> Haw.—Peace River, northern Alberta	New Mexico to California and north to Canada	± 66
*	<i>O. brasiliensis</i> (Willd.) Haw.	Brazil	22
Tribe Cereeae			
9	<i>Carnegie gigantea</i> (Engelm.) B. & R.	Arizona and northern Mexico	22
10	<i>Lophocereus schottii</i> (Engelm.) B. & R.	Northern Mexico	22
11	<i>Echinopsis multiplex</i> (Pfeif.) Zucc.	Brazil	22
†	<i>Zygocactus truncata</i> Schum.	Brazil	24
12	<i>Schlumbergeria russelliana</i> (Gard.) B. & R.	Brazil	22
13	<i>Echinocereus engelmannii</i> (Parry) Rump.	New Mexico to California and northern Mexico	44
14	<i>Ferocactus rostrii</i> B. & R.	Southeastern California	22
15	<i>Neomammillaria macdougalii</i> (Rose) B. & R.	Arizona	22
16	<i>N. applanata</i> (Engelm.) B. & R.	Texas	18
17	<i>N. fragilis</i> (Salm-Dyck) B. & R.	Mexico	22
†	<i>N. glochidiata</i> (Mart.) B. & R.	Southern Mexico	24

\* Counted by JOHANSEN (5).

† Counted by SUGIURA (9).

for the northernmost collection of cactus known. Of these, the northernmost form, much smaller than its more southern relatives and with smoother pads and fewer spines, had the somatic number 66



FIGS. 1-17.—Somatic chromosome plates from root tips of various cactus species. The numbers correspond to those of table I.

while the remainder had 44. This fact bears out the observation of HAGERUP (2): "It is worth noting that among these four pairs of species (including *Empetrum nigrum* and *E. hermaphroditum*) those with the higher chromosome numbers are always the ones growing farther north." But it is probable that in the *O. polyacantha* from Peace River the larger chromosome number merely enables the plant to grow locally where it could not otherwise survive.

Polyploidy with the ensuing greater possibilities for variation through changes in the chromosome set-up may furthermore enable a species to occupy a more extensive territory. Of the Cereaceae examined, seven had 22 chromosomes, one had 18, and one had 44. The geographical area of these plants is usually not so great as that of the opuntias, but it is interesting to note that *Echinocereus engelmannii*, with its various forms, has the greatest distribution and the largest chromosome number. Among the opuntias, *O. polyacantha*, the northern species with 44 and 66 chromosomes, is the most widely distributed of those here examined. *O. phaeacantha* also with 66 chromosomes is southern, but its geographical range is great. *O. discata* with the same number is restricted in area but it seems to be morphologically only a localized form of the widely distributed species *O. engelmannii*, of which no material was available. Of the two opuntias having 22 chromosomes, the range of *O. santa-rita* is limited while *O. chlorotica* is widespread but sporadic in its occurrence and it seems to be rather selective in its environmental requirements. These facts would indicate that there may be a correlation between distribution and polyploidy. This is in harmony with NAWASCHIN'S (6) statement that, "through change in the rate of development, a polyploid individual may acquire the ability of withstanding different climatic conditions. As a consequence it may penetrate into a new territory. . . ." This view is concurred in by HAGERUP (4), who states, "polyploid forms may be ecologically changed so as to grow in other climates and formations where the diploid form will not thrive." His work with desert plants (3) bears out this statement as in some cases polyploidy increased under increasingly adverse conditions.

Inasmuch as cacti range from considerably below the equator in South America to 56° north latitude, are epiphytic in the tropical

rain forests, and are among the few representatives of the plant kingdom in certain parts of the desert, it is expected that a more exhaustive research from this viewpoint may prove a valuable approach to the problems of plant distribution and the species question.

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## MATERNAL TRANSMISSION OF MUTATED PLASTIDS IN THE JAPANESE MORNING GLORY

KIICHI MIYAKE AND YOSHITAKA IMAI

(WITH TWO FIGURES)

Chlorophyll variegation, which is commonly found in the Japanese morning glory (*Pharbitis nil*), is transmitted as a simple recessive to the self-green condition. The whitish patches appear somewhat irregularly on the respective leaves of variegated individuals, so that the variegation is regarded as a pattern character. The allelomorphs, normal (self-green) and variegated, are quite constant and no cases of mutations either from normal to variegated or its reverse have come under our notice.

In 1928 three unexpected variants with variegated leaves incidentally occurred in the hybrid progeny of different crosses, their sister plants being invariably self-green. The variegation of these plants differed in color, namely:

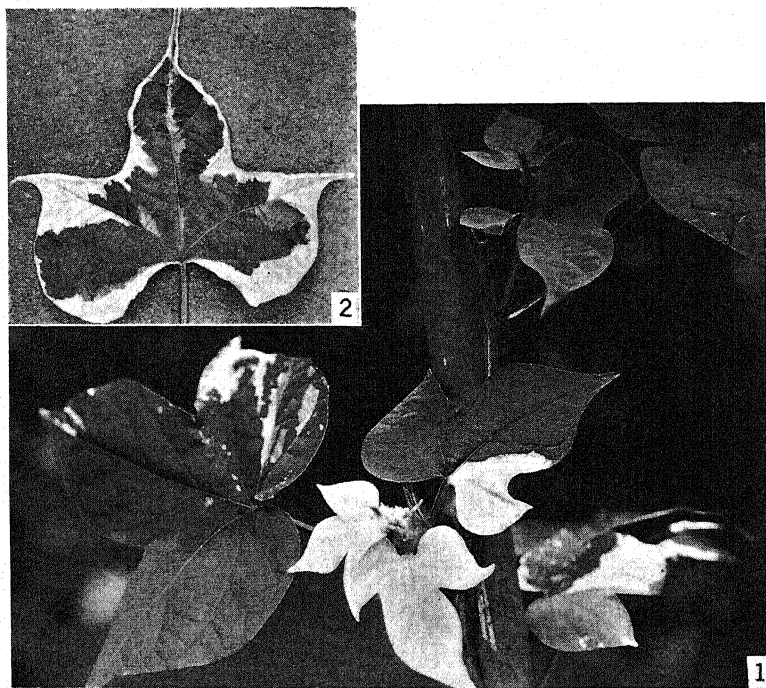
1. White; variegation in white.
2. Creamish; at first cream in color and creamish white in the extended leaves.
3. Yellowish; variegated with yellowish color, a little more intense in the young leaves.

The white-variegated variant puts out at times green and white branches (fig. 1) and also chimerical bud variations with periclinal white-over-green tissues. The results obtained from selfing the flowers of these branches are collected in table I.

The albinotic seedlings had short hypocotyls with small cotyledons and perished about two weeks after germination. The green individuals thus segregated bred true to type for generations, while the variegated ones gave rise to three forms in various proportions according to the amount of variegation of the mother plants or of the branches from which the seeds were collected. The slightly variegated branches or individuals gave mostly green seedlings, whereas the highly variegated ones produced mostly albinotic seed-



lings. The variegated segregates were at all events few in proportion compared with the green and white segregates. The chimerical branches shown in table I were of green "cores" with white "skins" (fig. 2); as expected, they produced only albinotic seedlings.



FIGS. 1, 2.—Fig. 1, white-variegated plant, with green (upper right) and white (lower left) branchlets. Fig. 2, chimerical leaf with green core covered with white skin, taken from progeny of the original variegated. The crumpling of the leaf is due to the gene crumpled-1.

At the same time the original variegated plant was crossed with a self-green strain in reciprocal ways, the results being given in table II.

Table II shows that the case is evidently non-Mendelian owing to plastid inheritance. Five green plants obtained by cross green ♀ × variegated ♂ were selfed and they bred true to type in the subsequent generation, as expected. In experimenting with the other variegated mutants, the same procedure was followed, when it was

found that both creamish and yellowish plastids were inherited maternally out of Mendelian rule. Up to the present a considerable number of cases of plastid inheritance have been studied, and recently DE HAAN (1) made a collection of available data to that date, to which he added his own criticisms.

The origin of the variegation in these variants of the Japanese morning glory is due to sporadic occurrences of plastid mutations.

TABLE I  
OFFSPRING OF WHITE-VARIEGATED VARIANT, SHOWN SEPARATELY  
ACCORDING TO TYPES OF BRANCHES

CHARACTER OF BRANCH	GREEN	VARIEGATED	ALBINOTIC	TOTAL
Green.....	69	.....	.....	69
Variegated.....	168	10	38	216
White.....	.....	.....	28	28
Chimerical.....	.....	.....	15	15

TABLE II  
OUT-BREEDING DATA OF WHITE-VARIEGATED VARIANT

CROSS	GREEN	VARIEGATED	ALBINOTIC	TOTAL
Green×white.....	66	.....	.....	66
White×green.....	.....	.....	24	24
Green×variegated.....	48	.....	.....	48
Variegated×green.....	25	3	19	47

Let us suppose that one of the green plastids, contained in an embryonic cell of the original white-variegated plant (variegation had appeared already at the seedling stage), had mutated to a white one and had a chance to propagate in a cell or cells of the growing point of the seedling. This is one of the most likely explanations to account for the origin of the variant. For the other two cases of variegation, similar mutations would take place, but from green to creamish or yellowish and possibly at different times of somatogenesis (variegation appeared later when the plant began to take on a luxuriant growth). In these cases the plastid mutations were sporadic in their occurrence (not mutable) and the mutated plastids never reverted to their prototype so far as we could observe. Prior to this, IMAI

(2) observed plastid mutations in this plant and wrote: "I have met with two or three cases, in which the variegated leaves or branches were produced on self-green plants, in my own culture. The appearance of variegation on the self-coloured leaves was due not to a factor mutation, but to a plastid mutation, their transmission, therefore, being non-Mendelian." In another publication (3) he gave a short note on the occurrence of the three forms of plastid mutations which we are investigating at present. In Asagao-Sô, a classical monograph of the Japanese morning glory published in 1817, we find two colored pictures of different specimens, each showing a mosaic of green and variegated colorations of leaves (2, figs. 6 and 7). Although, as pointed out by IMAI, the colors are somewhat exaggerated, the depiction of such mosaics is no doubt explained by the fidelity of the earlier authors to facts in their drawings and descriptions. Two possible explanations may be suggested to account for the origin of the mosaic specimens; namely, gene mutations occurring vegetatively from green to variegated, or plastid mutations from green to white. As the chances for the former seem more remote than the latter in this plant, we are inclined to accept the latter view also in these classical cases.

The expenses of the present research were partly defrayed by a grant from the Imperial Academy, to which we wish to express our cordial thanks.

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## CURRENT LITERATURE

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### Excretion in the higher plants

Following a suggestion of the late Professor KOSTYTSCHEW, FREY-WYSSLING<sup>1</sup> has written a monograph on the excretory and secretory processes of the higher plants. It is volume 32 of the *Monographien aus dem Gesamtgebiet der Physiologie der Pflanzen und der Tiere*. The products of assimilation and dissimilation are so numerous and varied that the work covers a wide range of material and many diverse processes. Even then the work will be found not to have covered all of the wastes and secretions that are formed in metabolism.

The author has not confined his attention strictly to the excretion and secretion phenomena, but surveys structural, physiological, and even phylogenetic problems that throw light on these processes of waste removal and deposition of metabolites. The work is the more useful and stimulating, however, for this well rounded and broad treatment.

The introduction sets forth the general relationship of such processes as absorption (resorption), assimilation, and dissimilation to excretion, secretion, and "recretion." True excretion follows dissimilation, while secretion is related to assimilation. Recretion is a term used to express the removal of absorbed (resorbed) mineral elements which are obtained from the soil, and which function in the active protoplasm for a time, but without being assimilated. These minerals are finally deposited by dehydration, precipitation, diffuse deposition in cell walls, or are removed by guttation.

There are four main sections to the work as follows: (1) cell wall; (2) recretion; (3) excretion; and (4) secretion. A brief summary, literature list, and subject index conclude the monograph.

In connection with the cell wall discussion, a great deal of attention is given to submicroscopic structures such as micellae, intermicellar spaces, and intermicellar deposits. The physiological significance of the walls also is considered at some length, such subjects as swelling of walls, their elasticity, plasticity, growth, and permeability and semipermeability relations being given detailed treatment.

The section on recretion deals mainly with the calcium salts (oxalate and carbonate) and silicic acid. Here again such physiological processes as absorption of minerals, ion exchange, selective absorption, and the inability of plants to exclude materials in the environment of the roots are brought in to widen the horizon of the discussion.

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<sup>1</sup> FREY-WYSSLING, A., *Die Stoffausscheidung der höheren Pflanzen*. 8vo. pp. xii+378. Springer, Berlin. 1935.

The excretions are all terpenes or their derivatives, such as isoprene, ethereal oils, balsams, camphor, resins and resin acids, crocetin, vitamin A, vetulin, sapogenin, carotenoids, and caoutchouc.

The last chapter, on secretions, considers the external secretions such as are produced by root hairs, haptotropic organs, nectaries, digestive glands, etc.; and some of the internal secretions, particularly the secretion of enzymes and hormones of various kinds.

Plants are in sharp contrast to animals in that most of their waste materials are deposited within their own bodies. Without important excretory organs, plants have met this problem of existence as effectively as animals. This monograph is a timely and valuable summary of a somewhat neglected field of plant physiology.—C. A. SHULL.

#### Statistical methods for research workers

The fifth edition of FISHER's<sup>2</sup> monograph appeared late in 1934, thus maintaining the policy of frequent editions of this work. This edition shows only a few changes from the preceding one. Section 5 is a historical note on the main contributors to statistical theory; two new sections, 21.01 on YATES' correction for continuity and 21.02 on the exact method for  $2 \times 2$  tables, have been inserted in the chapter on goodness of fit; and section 49.1 dealing with the analysis of covariance has been revised and slightly enlarged.

Section 29.1 at the close of the chapter on significance of means gives methods for the omission of an independent variate when it is found that such variate may with advantage be omitted. To be able to do this in a simple fashion should encourage workers to include more independent variates in their studies than has been customary in the past. If they are found to be insignificant, it is relatively easy to omit the useless variates after the calculations have been made, without complicated recalculations. The work is an extremely useful guide to the problems of biological statistical analysis.—C. A. SHULL.

#### Silviculture

The continued interest in the teaching and practice of silviculture is shown by the appearance within less than a year of two texts on the subject. The first<sup>3</sup> is really a third edition of a standard text rewritten and brought up to date. The earlier edition was noticed in this journal,<sup>4</sup> and its success is indicated by the demand for a revised edition after an interval of 15 years. While intended as a guide to technical foresters, it shows clearly the application of the principles

<sup>2</sup> FISHER, R. A., Statistical methods for research workers. 5th ed. 8vo. pp. xiii+319. Oliver and Boyd, Edinburgh and London. 1934.

<sup>3</sup> HAWLEY, R. C., The practice of silviculture with particular reference to its application in the United States of America. 3d ed. 8vo. pp. xv+340. figs. 67. John Wiley & Sons, New York. 1935.

<sup>4</sup> Bot. Gaz. 87:668-669. 1920.

of ecology to the development of economic forest. A desirable feature of this text is the list of references at the close of each chapter that may serve to guide the reading of the forester.

The second volume<sup>5</sup> is somewhat broader in its scope and embraces both the theory and the practice of forest production. The five parts into which the book is divided indicate the breadth of its scope. They are: plant physiology, forest ecology, systematized silvicultural experience, the forest itself as a source of silvicultural knowledge, and silvicultural literature. The earlier parts analyze the factors which constitute forest environment and the latter apply the results of this analysis to the problems of forest production. Both books have come from ripened experience in two of our leading schools of forestry, and will prove valuable aids to teachers and students.—G. D. FULLER.

### The Botanical Review

A botanical magazine<sup>6</sup> edited and published with a new purpose and on a new plan has made its appearance. It is to be issued monthly and the first number appeared in January, 1935. Each issue is to contain two or three articles summarizing and evaluating all recent important contributions in that particular portion of the botanical field. Eventually all phases of botany will be considered. Each article will be written by an expert authority at the invitation of the editors, unsolicited manuscripts not being accepted. The yearly volumes will consist of about 400 octavo pages.

The journal is owned and edited by H. A. GLEASON and E. H. FULLING of the New York Botanical Garden, who have associated with them a board of advisory editors whose scientific attainments guarantee a high standard for the journal. It is printed by the Science Press Printing Company.

The January and February issues contain: Possibilities in plant virus classification, by L. O. KUNKEL; The structure of protoplasm, by WILLIAM SEIFRIZ; Glacial and postglacial vegetation, by PAUL B. SEARS; The structure of the walls of higher plants, by DONALD B. ANDERSON.

It would seem that such a journal should have a wide circulation and prove particularly useful to botanists who have access to only limited library facilities. It should certainly prove time-saving to all.—G. D. FULLER.

### Day lilies

Some of these attractive plants, species of *Hemerocallis*, have become so completely naturalized in parts of the United States that they are almost regarded

<sup>5</sup> BAKER, F. S., Theory and practice of silviculture. 8vo. pp. xiv+502. figs. 87. McGraw-Hill, New York. 1934. \$5.

<sup>6</sup> The Botanical Review. H. A. GLEASON and E. H. FULLING, editors and publishers. New York Botanical Garden, Bronx Park, New York and Lime & Green Streets, Lancaster, Pa. \$3.00 per year; issued monthly.

as natives. STOUT,<sup>7</sup> however, is convinced that all truly wild day lilies are confined to Asia, where their range is from Manchuria to the Caucasus. Probably other wild species, hitherto unknown, may be brought from the Orient into cultivation and added by plant breeders to the existing horticultural varieties.

The present volume describes both the botanical species and the cultivated varieties. It is illustrated by numerous plates, several of them in color, and many from the larger collection grown in the New York Botanical Garden. An unusual feature of the book is the brief biographical sketches, together with the addresses, of the principal plant breeders and florists who have hybridized and developed these plants.—G. D. FULLER.

#### Botany in Poland

A recent sketch<sup>8</sup> of the history of the development of botany in Poland will tend to give that country the recognition it deserves. With the usual beginning with the herbals of the 16th century, six different periods are recognized. Sketches of the contributions of individual botanists are enlivened by portraits, beginning with that of SZYMON SYRENSKI (SYRENIUS), 1540-1611, and ending with those of living botanists. Emphasis is placed on the rapid advancement made since 1918.—G. D. FULLER.

#### Moss flora of North America

The final part of GROUT's work<sup>9</sup> on the pleurocarpous mosses (volume III) appeared recently. Parts 1<sup>st</sup> and 2<sup>nd</sup> have already been reviewed in this journal. Besides completing the family Leskeaceae, part 4 also contains the families Hypopterygiaceae, Hookeriaceae, Neckeraceae, Meteoriaceae, Pterobryaceae, Leucodontaceae, Cryphaeaceae, Fabroniaceae, and Fontinalaceae.

The genera *Pseudoleskea* and *Leskea* of the family Leskeaceae were studied by A. J. SHARP, and the family Fontinalaceae by WINONA H. WELCH. In the latter monograph WELCH has constructed keys based chiefly upon vegetative characteristics, a valuable feature since the water mosses are often collected when not in fruit.

Adequate keys to the genera of each family and to the species of each genus are provided. Synonyms, descriptions, citations of previous illustrations, exsiccati, and varieties are given for each species. A complete index to the

<sup>7</sup> STOUT, A. B., Day lilies. The wild species and garden clones, both old and new, of the genus *Heemerocallis*. pp. vi+119. pls. 36. Macmillan, New York. 1934. \$3.

<sup>8</sup> HRYNIEWIECKI, BOLESŁAW, Précis de l'histoire de la botanique en Pologne. pp. 45. 57 portraits. Publ. by Soc. Bot. Pologne. Warsaw. (1931) 1934.

<sup>9</sup> GROUT, A. J., Moss flora of North America north of Mexico. Vol. III. Pt. 4. 4vo. pp. 179-277. pls. 45-80. Newfane, Vermont. Published by the author. 1934.

<sup>10</sup> BOT. GAZ. 88:111. 1929.

<sup>11</sup> BOT. GAZ. 93:110-111. 1932.

families, genera, species, and varieties covered by volume III is included with this part.

The aid of SEVILLE FLOWERS in illustrating numerous species is acknowledged by the author. None of the excellent line-drawn plates are duplications of illustrations in GROUT's previous publication, *Mosses with Hand-lens and Microscope*.

Students of American mosses, as well as those who collect mosses as a hobby, will welcome the completion of this volume since it combines authenticity and convenience, and supplies what the older manuals, now out of print, cannot, a summary of our most recent knowledge of the mosses of the greater part of this continent.—P. D. VORH.

#### Ferns of the northwest

In this volume,<sup>12</sup> both the lycopods and the ferns proper are included under the general title of ferns; and the northwest covers Washington, Oregon, Idaho, British Columbia, Montana, Wyoming, central and northern California. Taxonomic keys, a full bibliography, and a glossary make it easy for the layman to identify his local flora. The book is more than a taxonomic manual; for more than 30 years the author tramped the entire region covered by the work, and his field observations are recorded throughout. Even the taxonomic keys give the impression of the field rather than of the herbarium sheet. Many habitats are given, especially for Washington and in cases of rarer species. Throughout the book there are observations on variation which raise the question whether the differences are due to genetic relationship or to ecological conditions. The book will be useful to both the professional botanist and the layman who would know more about the ferns. It is copiously illustrated, by both drawings and photographs.—C. J. CHAMBERLAIN.

#### Illumination and growth

A recent study<sup>13</sup> has been made of the relation of various intensities of illumination to growth in the radish. The author grew plants in tents having one-ninth, one-third, one-half, three-fourths, and full daylight. She finds that the ratio of water to dry substance at most ages tends to decrease as the light is more intense; in other words, the percentage of dry matter is greater when plants are grown with strong illumination. Thus at one month under the intensities named the total dry weight percentages calculated from her figures are 4.8, 6.1, 7.5, 7.7, and 8.4. Not only are actual dry weight and dry weight percentage greater, but the mineral matter likewise increases with the illumination, increased light favoring absorption of mineral nutrients, as has already been

<sup>12</sup> FRYE, T. C., *Ferns of the northwest*. 8vo. pp. vi+178. Metropolitan Press, Portland, Oregon. 1934. \$1.75.

<sup>13</sup> PANCHAUD, J., *Action du milieu extérieur sur le métabolisme végétal*. *Rev. Gén. Bot.* 46:586-603. 1934.



shown by the work of others. For growth in length and surface of body organs it is found that the lower illuminations are more favorable during early seedling stages while for older stages the higher light intensities serve best. With the faint illumination of one-ninth the radish plants spread their cotyledons early but produced no or few foliage leaves.—FRANCIS RAMALEY.

#### Wild flowers

Botanists and plant lovers in general will welcome a recent book.<sup>14</sup> It is essentially a reprinting in one volume of HOUSE's beautiful and well known work,<sup>15</sup> now practically unavailable. Aside from the addition of two new photographs in halftone and a rewritten introduction, the text and illustrations are the same in the two editions. The material has been compressed into one book through the use of lighter stock, and through the utilization of both sides of each page in the printing of the color plates. These 364 half-page and full-page color illustrations of the more striking herbaceous plants of northeastern United States have long been known as masterpieces of the photographers' and printers' arts. Issued under the auspices of a well known publishing house, this work should now have a wider circulation, and should do much to stimulate the layman's interest in our native plants.—C. E. OLMSTED.

#### Forest mensuration

Professional foresters and students will find this book,<sup>16</sup> the third to appear in the American Forestry series, a distinct contribution to forestry literature, owing to the fundamentally new method of approach. Modern methods in statistics and graphics are adapted and utilized to the fullest extent in showing how problems in measurement of timber crops may be solved. Numerous illustrative examples, as well as exercises and problems at the close of each chapter, enhance the value of the work as a textbook. Workers in other fields of biology in which biometrical methods are used will also find features of interest.—C. E. OLMSTED.

<sup>14</sup> HOUSE, HOMER D., *Wild flowers*. 4to. pp. 362. pls. 364. Macmillan, New York. 1934. \$7.50.

<sup>15</sup> ———, *Wild flowers of New York*. Vols. I, II. Memoir 15, New York State Museum. pp. 362. Illus. Univ. of State of N.Y., Albany. 1918.

<sup>16</sup> BRUCE, DONALD, and SCHUMACHER, F. X., *Forest mensuration*. 8vo. pp. xiv+360. figs. 102. McGraw-Hill, New York. 1935. \$3.50.

# THE BOTANICAL GAZETTE

*June 1935*

## EFFECTS OF TEMPERATURE ON GROWTH, ANATOMY, AND METABOLISM OF APPLE AND PEACH ROOTS<sup>1</sup>

G. T. NIGHTINGALE

(WITH ELEVEN FIGURES)

### Introduction

Several publications have recently appeared in which effects of temperature were emphasized in their relation to the growth and metabolism of the aerial organs of plants (18, 22, 23). The studies here recorded are concerned principally with influences of temperature on the growth, anatomy, and metabolism of the roots of apple and peach. The results were obtained through simultaneous studies of progressive changes in composition and anatomy as they occurred in the growth of the roots concerned at the respective temperatures employed.

The value of anatomy in the interpretation of macrochemical analysis and, conversely, of chemistry in interpreting anatomical responses, was repeatedly demonstrated. Much apparent aberrancy in the interpretation of the results of macroanalysis of plant organs may well be due to lack of knowledge of the anatomical development of the material analyzed.

<sup>1</sup> Through the courtesy of the University of Chicago there was made available for this work the temperature control equipment and laboratory facilities of its department of botany. The writer wishes particularly to acknowledge the advice and generous co-operation of E. J. KRAUS and M. A. BLAKE.

Journal series paper of the New Jersey Agricultural Experiment Station.

## Experimental methods

## APPLE TREES

The apple trees used in these experiments were root grafts of the Stayman variety, and when received from a commercial nursery were 45-50 cm. in height. About 400 trees were selected for uniformity from a much larger population, and on December 1, 1933, after the dormant trees had been several weeks in a cold storage cellar, they were washed free of soil and all the fine fibrous roots removed so that there remained only old roots, none of which was less than 1 cm. in smallest diameter. The tops were also cut back so that when planted they were 30 cm. in height. This left several buds.

TABLE I  
COMPOSITION OF NUTRIENT SOLUTION  
PARTIAL VOLUME MOLECULAR CONCENTRATION OF SALTS USED

	$\text{Ca}(\text{NO}_3)_2$	$\text{KH}_2\text{PO}_4$	$\text{MgSO}_4$	$\text{CaCl}_2$
Complete or plus- $\text{NO}_3$ ..	0.0090	0.0045	0.0045	.....
Minus-N.....	.....	0.0045	0.0045	0.0090

Only one of these, the most distal bud, was allowed to remain; otherwise the number of growing points per tree would have been variable, thereby making more difficult the determinations of amount and quality of growth.

The trees were then set four to a 12-liter, self-draining porcelain jar. Some coarse glass wool was placed over the drainage outlet in the center of the bowl-shaped bottoms and the jars were nearly filled with nitrogen-free white quartz sand, which had been sifted so that all particles passed through a 1.0 mm. sieve and were held by a 0.5 mm. sieve.

Until January 31, 1934, the trees remained in the culture jars in a storage room at 35°-38° F. (all temperatures herein mentioned are Fahrenheit) and received an application of minus-N solution (table I) once a week in sufficient quantity to flush the sand. The cultures were kept constantly moist by the application of tap water as required. At that time, 30 trees (hereafter referred to as initial trees)

were harvested for chemical analysis. The remainder of the trees in the porcelain jars were shifted to water baths, as described by LINK,<sup>2</sup> which were thermostatically regulated to  $\pm 0.2^\circ$  to give the respective sand temperatures indicated in table II. At each tempera-

TABLE II  
ELBERTA PEACH TREES  
AVERAGE CURRENT GROWTH PER TREE AT DIFFERENT SAND TEMPERATURES

SAND TEMPERATURE (°F.)	FRESH WEIGHT IN GRAMS						LENGTH IN CENTIMETERS		
	ROOTS			STEM AND LEAVES			STEM		
	MAR. 17	APRIL 4		MAR. 17	APRIL 4		MAR. 17	APRIL 4	
		—N	+NO <sub>3</sub>		—N	+NO <sub>3</sub>		—N	+NO <sub>3</sub>
		—N	—N		—N	—N		—N	—N
45.....	None	None	0.2	0.7	2.0	2.0†	0	0	0
50.....	0.3	4.5	5.0	1.5	3.0	9.0	5	7	12
55.....	7.0	7.0	7.0	5.5	6.0	14.0	12	25	28
60.....	8.5	11.0	13.0	7.5	7.5	14.5	20	25	33
65.....	8.0	12.0	15.0	8.6	11.5	21.0	23	30	41†
75.....	5.0	7.0	6.0	4.7	8.0	19.0	18	23	38
85.....	2.0	2.2	2.0	3.5	6.0	7.2	12	18	25
90.....	0.4	0.2	0.2—	3.2	3.5	5.0	7	10	15
95.....	None	.....	.....	0.7	.....	.....	5	.....	.....
60-45*	8.5	9.2	9.8	7.5	8.6	9.0	20	23	25
60-95*	8.5	7.0	6.0	8.5	8.8	9.0	20	33	38
90-60*	0.4	2.0	1.5	3.2	7.0	5.0	7	12	12
45-60*	None	8.9	9.5	0.7	5.0	7.2	0	10	15

\* Shifted March 17.

† In addition to 41 cm. of primary current stem there was lateral stem growth which alone was 44 cm. in length.

ture there were employed 36 trees. The cultures were each provided with independent drainage by means of a tube with watertight connections which ran with a downward slope from the outlet in the bottom of each jar out through the side walls of the temperature bath. Here the drip from the cultures was frequently collected for determinations of pH values. The temperature of the air in the greenhouse was in all cases held at approximately 60°-65° at night and 65°-70° during the day.

In the following discussion all references to temperature, unless

<sup>2</sup> LINK, G. K. K., Science 81:204-207. 1935.

otherwise indicated, will refer to the temperature of the sand in the culture jars.

With a few exceptions, to be mentioned elsewhere, all of the apple trees received daily applications of the complete or plus- $\text{NO}_3$  solution (table I) in sufficient quantity to flush the sand, and once a week each culture was washed with several liters of distilled water after which nutrient solution was immediately applied. Particularly at the higher temperatures, the sand in the culture jars dried out to a depth of 1 or 2 cm. This dry surface layer apparently acted as a mulch, however, as even at  $95^\circ$  there was always an abundance of water in the sand at lower levels.

#### PEACH TREES

About 400 uniform "one-year-old" Elberta peach trees 90-100 cm. in height were also used in these experiments. They were pruned back to a height of 30 cm. and otherwise handled in the same manner as the apple trees, except as to nutrient treatment. When shifted from cold storage to the temperature baths on January 31, all of the peach trees continued to receive the minus-N solution until March 17. On that date and until finally harvested, some of the peach trees were supplied with the complete or plus- $\text{NO}_3$  solution and others continued to receive the solution lacking nitrogen (table I). The amount and frequency of application of solution, however, were exactly the same as already described for the apple trees. Ferrous sulphate was applied to the peach trees to avoid iron deficiency (13). This was not necessary in case of apple. Boron and manganese were present in sufficient amount either in the salts applied or in the tissues of the initial trees of both peach and apple.

On March 17, some of the cultures of both apple and peach were shifted from one sand temperature to another, as indicated in table II.

Methods of chemical analysis have been described in recent publications (4, 22). The current fibrous roots from ten or more trees were removed and analyzed macrochemically as a single sample. The old large lateral roots from the same trees were employed as a second analytical sample. They varied from 0.5 to 1.5 cm. in

diameter and were from 8 to 10 cm. in length (figs. 1-3). Macro-analyses of other parts of the trees are not reported in detail.

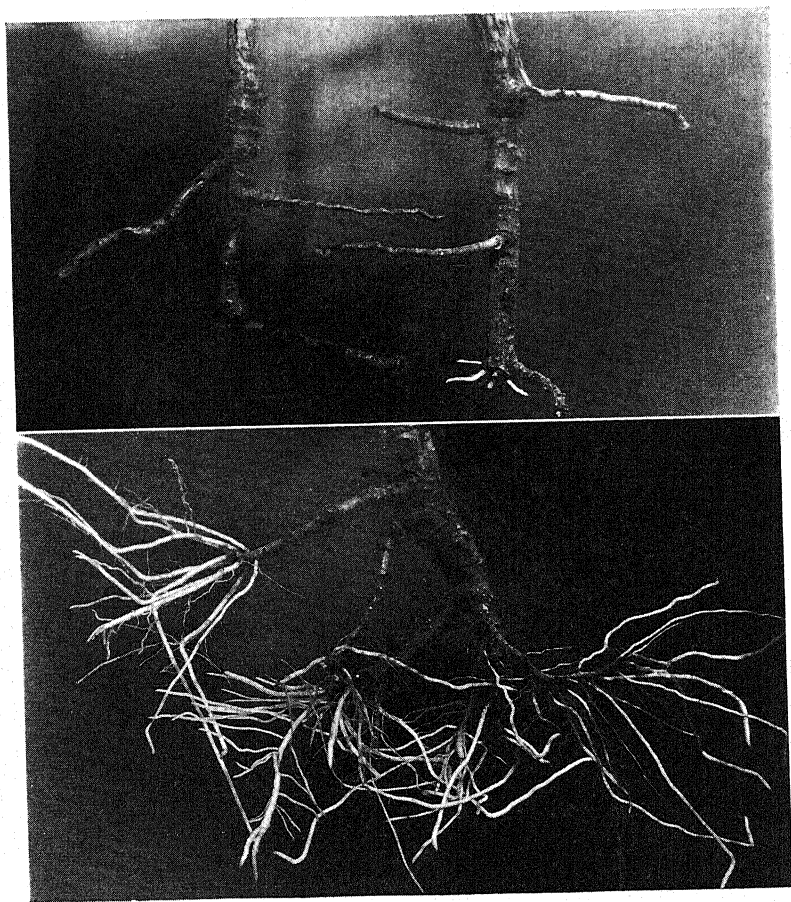


FIG. 1.—Peach roots March 17. Upper left, 45°; upper right, 50°; lower illustration, 55° F. Note that there are no new roots at 45°, few at 50°, and at 55° many rather short, white, typically large-diameter succulent roots with few fine laterals.

ECKERSON'S (6) method was employed for reducase determinations, except that instead of adding 9 cc. of distilled water to 1 cc. of standard nitrite solution there was added 9 cc. of nitrite-free extract from an aliquot of the tissue to be tested for reducase activity.

The extracts were invariably yellowish brown in color and this method of preparing the standard solution facilitated the making of color comparisons.

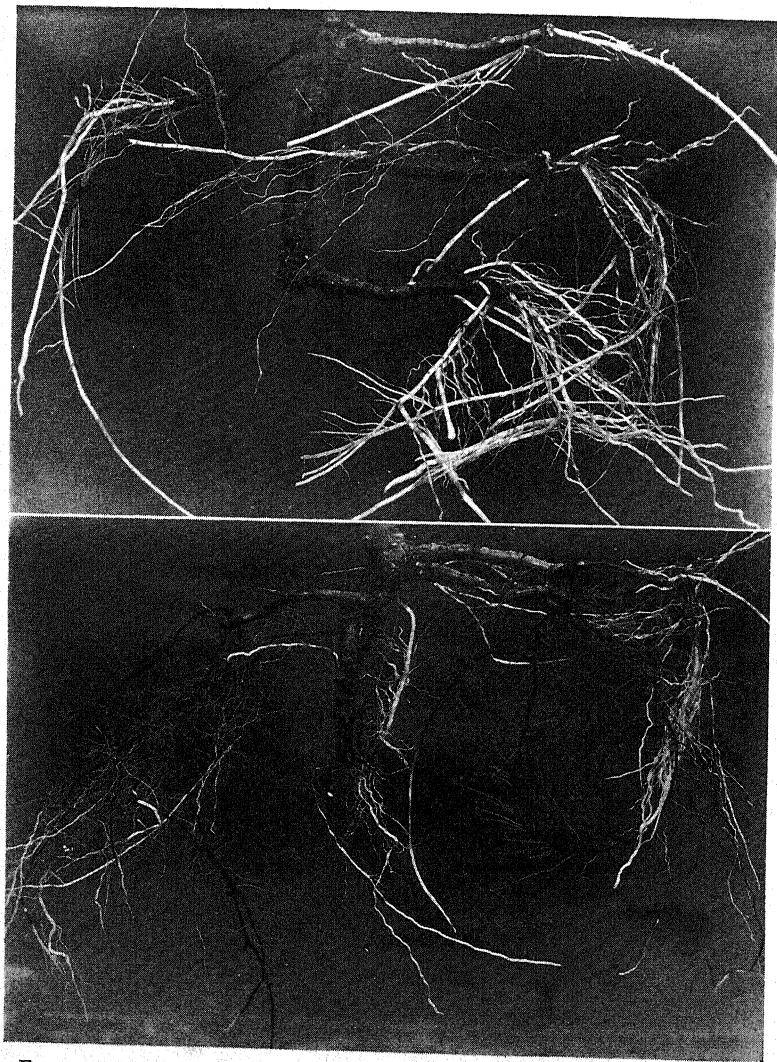


FIG. 2.—Peach roots March 17. Upper illustration, 65°: current roots more extensive but similar in quality to those at 55°; lower illustration, 75°: note dead cortex and fine laterals. These roots lacked succulence.

The usual microchemical methods (9) were followed and the reagents and range indicator method described by SMALL (27) were used in determining the pH values of various plant tissues.

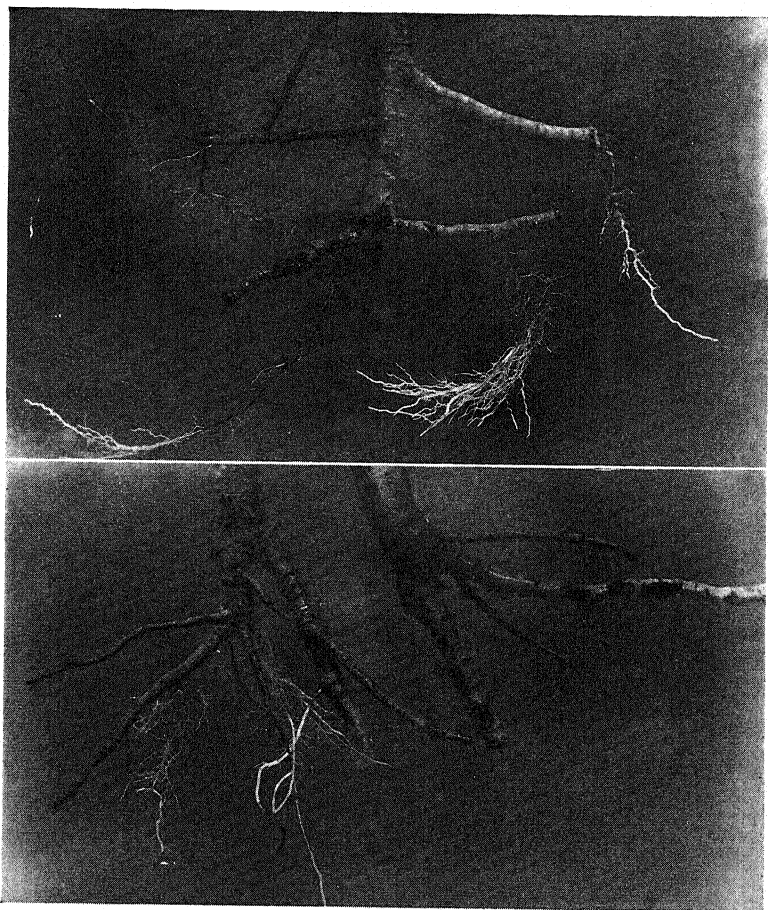


FIG. 3.—Peach roots March 17. Upper illustration, 85°: limited development of non-succulent current roots of very small diameter with short-lived cortex and numerous fine laterals; lower left, 90°: roots much like those at 85° but less extensive; lower right, 95°: no new roots developed.

In preparing permanent slides, already well recognized methods were employed that need not be described in detail. Material was fixed in Navashin's solution, dehydrated, imbedded in paraffin, sec-



tioned, and stained with safranin, gentian violet, and gold orange. All drawings were made on the same scale with the aid of a projectoscope.

The pH estimations of nutrient solutions were made colorimetrically using as standards the colored glass discs of a Hellige Klett color comparator. Solutions were tested before and after passing through the sand of the cultures, necessary adjustments being made with 0.1N KOH or  $\text{H}_2\text{SO}_4$  as required. The solutions as applied were approximately pH 4.5 but after passing through the sand they frequently reached a pH value of 5.8-5.5. These conditions are such, however, as to permit good growth and fairly vigorous assimilation of nitrate (3, 19, 31, 32) if other environmental factors are favorable.

### Discussion of results

#### EXTERNAL APPEARANCE OF APPLE AND PEACH ROOTS AT DIFFERENT TEMPERATURES

When the initial apple and peach trees were shifted from cold storage to the respective temperature baths on January 31, they had no fibrous roots, no callus tissue had developed on the pruning wounds of the large roots, and the buds had not enlarged noticeably. The trees were therefore comparatively inactive when experimental treatments were commenced. The results reported here are consequently in contrast to those recently (19, 22, 23) secured with actively growing fruit trees which were shifted to various extremes of temperature when they had initially present a well developed system of current fibrous roots and tops.

On March 17, after the cultures had been for 45 days at the respective sand temperatures indicated in table II, photographs were taken of typical root systems of peach (figs. 1-3) and average weights were recorded for both peach (table II) and apple (fig. 11). It is apparent that in both genera the greatest amount of roots was produced at 65°. In both cases the current roots weighed slightly less at 60° and 75° and still less at the greater extremes of high and low temperature. The minimum soil temperature at which roots will grow undoubtedly varies, however, not only with the genus but with the variety (15, 24). ROGERS (25) reports that in England actively

growing roots were found on pear, gooseberry, and black currant in February, 1930, while the tops were still dormant. It is likewise recorded in a recent report (19) that the initially present fibrous roots of Delicious apple trees increased materially in volume at  $48^{\circ}$  although there was practically no growth of tops at that temperature. GOFF (11) found that the root growth of many perennial plants started considerably in advance of top growth.

At  $45^{\circ}$  a few new fibrous roots appeared on the apple trees, all of which it will be recalled received the complete or plus- $\text{NO}_3$  nutrient solution (table I). A similar response occurred in case of all the peach trees with plus- $\text{NO}_3$  treatment at  $45^{\circ}$ , but none of the peach trees at this temperature which lacked an external nitrogen supply formed any new roots during the period of these experiments. This point may be of considerable practical value and will be discussed elsewhere in connection with reductase activity and effects on peach of added nitrate at the other sand temperatures employed.

When the peach trees at  $60^{\circ}$  with a well developed system of new roots were shifted to  $45^{\circ}$ , however, there was some growth of fibrous roots in case of both nutrient treatments (table II). The same situation also prevailed in case of apple when shifted from  $60^{\circ}$  to  $45^{\circ}$ .

At  $95^{\circ}$ , the highest temperature employed, there were no new roots produced in either genera. It has been shown (22, 23), however, that the initially present current fibrous roots of various varieties of apple and peach survived this temperature for a period of several days, although the roots were injured internally more or less seriously, depending upon the variety. As in the previous case, the peach and apple trees did not lose their fibrous root system, although the cortex died almost at once when shifted from  $60^{\circ}$  to  $95^{\circ}$  for a period of 18 days. Also the roots in a few days exhibited internally other characteristics which, as will be shown later, are typical of high soil temperatures.

Before leaving the subject of gross weight or volume of apple and peach roots produced, particular attention is called to the effects of comparatively small differences in temperature. At  $85^{\circ}$ , for example, the root systems of both genera were extremely small, yet at  $75^{\circ}$  there was in both cases a large volume of roots. Note also the amount of roots produced respectively by apple and peach at  $50^{\circ}$

as compared with  $55^{\circ}$ , and the roots produced at the latter temperature in relation to those developed where the sand was maintained at  $65^{\circ}$  (figs. 1-3, table II). It would seem apparent that the amount of root growth can be greatly modified by only a very few degrees' difference in the temperature of the soil.

Although the amount of root growth at different soil temperatures is important, at least of equal significance is the character or quality of roots produced. Note the peach roots produced at temperatures below  $75^{\circ}$  as shown in figures 1 and 2 on March 17. (And at comparable temperatures the apple roots were essentially the same, except that they started root growth a little later.) The roots were glistening white and typically of relatively large diameter. The cortex was still alive, as indicated by the fact that practically none of the roots were brown, even at their older or proximal end. A further characteristic of these roots was their extreme succulence; a comparatively slight bending caused them to break completely in two. On the other hand, at  $75^{\circ}$  and higher the current roots lacked succulence, except near the tips. The extreme toughness of these recently developed but non-succulent roots is evident from the fact that they could be tied in a knot without breaking. Figures 2 and 3 show also that even on March 17 much of the cortical tissue was brown and dead at  $75^{\circ}$  and higher.

That the current top growth of both apple and peach was closely correlated with the volume of roots produced, is shown by figure 4 and by the weight and linear growth of tops as recorded in table II. At the sand temperatures  $45^{\circ}$ ,  $50^{\circ}$ ,  $90^{\circ}$ , and  $95^{\circ}$ , the leaves were somewhat curled (fig. 4), undoubtedly owing to inability of the trees to absorb an adequate amount of water in the practical absence for several weeks of a fibrous root system.

In case of the peach and apple trees which on March 17 were shifted from  $60^{\circ}$  to  $45^{\circ}$  and to  $95^{\circ}$  respectively, there was present of course a well developed fibrous root system. These trees apparently absorbed water freely at  $45^{\circ}$  and  $95^{\circ}$ , as indicated by the fact that the tops of the trees of neither genus showed any sign of curling or wilting. The extremes of temperature evidently permitted intake of water.

As already mentioned, however, no fibrous roots developed and

none were present on any of the fruit trees continually at  $95^{\circ}$ , nor on those lacking nitrate and continuously at  $45^{\circ}$ . Nevertheless the tops of the trees did not die nor did the current top growth wither,

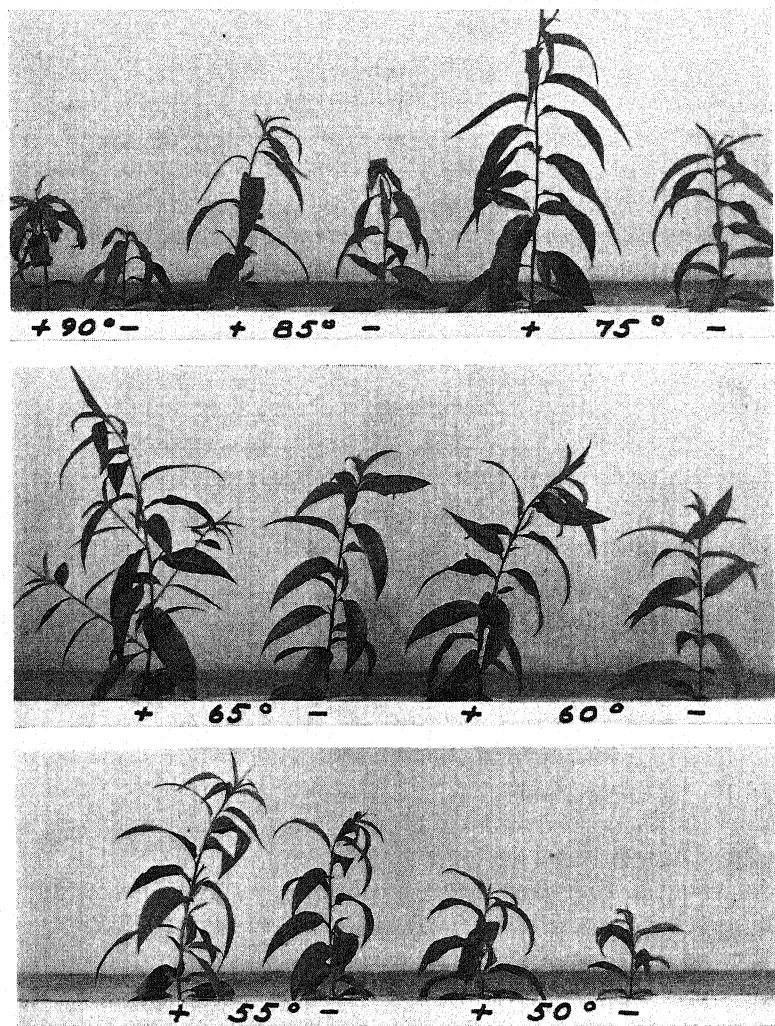


FIG. 4.—Current top growth of peach April 4. All tops grown at about  $65^{\circ}$ ; roots at temperatures indicated. The plus- $\text{NO}_3$  solution is designated by the plus sign and the minus-N solution by the minus sign. Note that in the case of each nutrient series the greatest growth occurred at  $65^{\circ}$  (cf. fig. 11 for apple).

although there was often slight wilting at mid-day and the leaves were continually more or less curled. There must obviously have been considerable absorption of water through the old large roots. At 95° many of the large roots of both peach and apple died, but up until March 17, when the trees at this temperature were discarded, the leaves had not become seriously wilted. In harmony with these observations, there is increasing evidence in the literature (14, 16, 2) that plants may obtain water through a dead root system and through the large roots. CRIDER (2) even reports selective absorption of ions by the large non-fibrous roots of *Citrus* and *Vitis*. It may be said here, however, that nitrate was found only in the current fibrous roots of peach and apple. There was no nitrate in the old large roots either living or dead under any of the conditions of these experiments.

The obvious ability of trees to live for a considerable period in the absence of a living system of fibrous roots is a fact which should be given serious consideration. The writer has frequently found apple and peach trees in commercial orchards that were annually under adverse conditions for weeks at a time and were during that period practically devoid of living fibrous roots. Effects on the tops were often quite gradual, however, and to the casual observer not noticeable for several years.

The various causes frequently resulting in death of the fibrous roots will not be discussed here, yet it may be pointed out that effects of mineral deficiency, drought, unfavorable pH of soil, etc. are apparent in the roots of fruit trees long before the tops are seriously affected. It may also be pertinent to recall that the fine fibrous roots are the initial seat of protein synthesis in apple (22, 30) and probably in peach (3, 23).

It should be remarked, however, that brown or dead cortex does not necessarily mean a dead central cylinder. Young fibrous roots in the orchard were commonly found to be white under cool soil conditions, but the cortical tissue was invariably short lived and soon turned brown at soil temperatures above 70°. The cortex is a juvenile tissue, of course, but may remain alive and white for weeks if the soil temperature is 65° or lower. These observations in the field are therefore in apparent agreement with those already re-

corded as occurring under controlled conditions, and will be further discussed in connection with the internal structure of roots as affected by temperature.

Before considering the very different anatomical structure of the roots of the respective temperature series, certain facts concerning the relative development of callus tissue may be recorded. During the two months' period of these experiments there was practically no callus development at temperatures at  $55^{\circ}$  and below, in case of either peach or apple, although over a period of several months it probably would have occurred (26, 29, 33). Wound callus, however, developed rapidly at higher temperatures. It was formed earliest at  $85^{\circ}$  and  $90^{\circ}$ . At the latter temperature there was incomplete covering of the pruning wounds and the callus became completely suberized when only a few cells thick, at which time practically all cell division had ceased. There was, in general, at  $85^{\circ}$  complete covering of pruning wounds by a moderately thick layer of callus which suberized and turned brown very rapidly. At  $75^{\circ}$  the wound callus tissue was thicker and remained white for a somewhat longer time. At  $65^{\circ}$  and lower the callus tissue failed to mature. It was white and fluffy in appearance, broke off easily in handling, and the white masses of proliferating meristematic tissue continued to enlarge until the trees were harvested on April 4. There was in this respect little, if any, difference between peach and apple.

Perhaps of practical significance in propagation is the fact that the pruning wounds of the roots of peach and apple callused over more or less satisfactorily at  $85^{\circ}$ , a temperature at least  $10^{\circ}$  higher than that which could be considered favorable for root development under the conditions of these experiments. Contrary to popular opinion, roots were not observed to originate from callus tissue except in an occasional instance.

At  $95^{\circ}$  no wound callus developed, although the lenticels on living portions of the old roots became very conspicuous owing to the development of callus growth that soon suberized. This was also true at  $90^{\circ}$  and  $85^{\circ}$ , at  $75^{\circ}$  to a lesser degree, while below  $75^{\circ}$  lenticel callusing was slight. Lenticel callusing was much more conspicuous in peach than in apple. Lenticel hypertrophy seems, therefore, to be favored by an even higher temperature than that which per-

mitted abundant development of wound callus and a much higher temperature than that which permitted good growth of roots.

There appears to be nothing in the literature bearing directly on these results, although work with cuttings of apple (26, 29) and peach (34) is in harmony with the preceding observations. There is also some evidence (26, 29) to indicate that wound callus and especially lenticel callus is not retarded, but possibly even favored by a somewhat limited oxygen supply. For root development there seems to be required an abundance of oxygen, especially at higher temperatures. All the cultures of these experiments were provided with facilities for aeration as described under experimental methods. Nevertheless, as will be explained elsewhere, there were apparently high concentrations of carbon dioxide in the tissues of the roots at 85° and higher, which aside from direct effects must have meant less available oxygen.

#### GENERAL ANATOMICAL STRUCTURE OF PEACH AND APPLE ROOTS

At 75° there was apparently present in both peach and apple roots the full quota of primary and secondary tissues. At other temperatures employed certain tissues did not develop or were limited in development, as will be shown later. The general structure of peach roots may therefore be most readily understood by reference to figure 5, which shows typical cross sections of young and relatively old portions of a current fibrous root as it developed at 75°. Corresponding drawings for apple roots are not shown as they were essentially the same under similar conditions.

Both genera possessed a well developed root cap, and at 1 cm. from the root tip the central cylinder was sharply differentiated from the cortex by a distinct endodermis with remarkably large casparian strips. These thickenings of the radial walls were characteristic of apple as well as peach. The cells of the root tip and those immediately adjacent were compact and angular, but the cortical cells only 1 cm. from the tip were somewhat rounded and small intercellular spaces were evident (fig. 5 A). Often the peripheral cells of the cortex were somewhat thicker walled just within the single layer of relatively small epidermal cells.

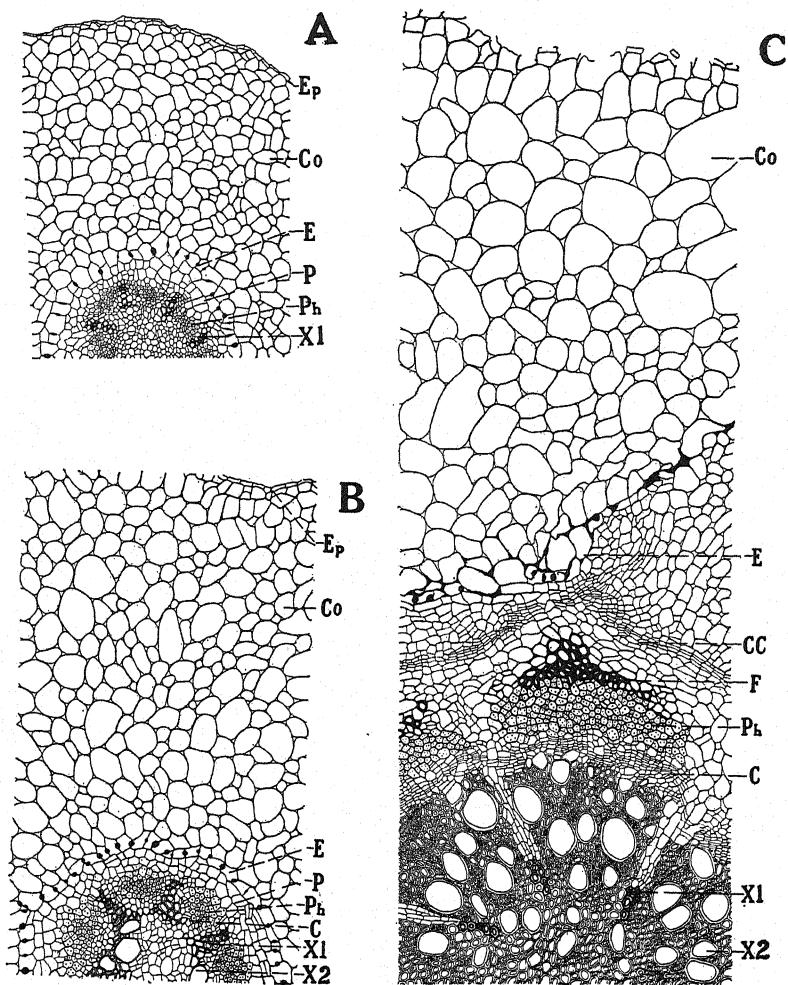


FIG. 5.\*—Transverse sections of peach root grown at  $75^{\circ}$ : A, 1 cm. from root tip. Cortical cells not fully expanded. There are evident dividing endodermal cells, partially thickened casparian strips, primary phloem, protoxylem points, and in center of stele closely packed, thin walled cells. B, 5 cm. from root tip. Outer cortical cells frequently dead, although endodermis, through cell division, has accommodated itself to development of secondary phloem and xylem. Note cell division in pericycle region. C, 11 cm. from root tip. Entire cortex dead, strong cork cambium, heavy walled pericycle, and phloem fibers. Note portion of lenticel on right, extensive development of secondary phloem and strongly lignified xylem.

\* Abbreviations for figs. 5-9: *Ep*, epidermis; *Co*, cortex; *E*, endodermis; *CC*, cork cambium; *P*, pericycle; *F*, fibers; *Ph*, phloem; *C*, cambium; *Xr*, primary xylem; and *X2*, secondary xylem.



At this point and somewhat farther back there were occasionally observed a few root hairs in both genera. They were of the usual type of modified epidermal cell about the length of three or four cortical cells. Many of the roots apparently had no root hairs. They occurred so infrequently under the cultural conditions of these experiments and have been observed so seldom in the field that no definite conclusions may be drawn concerning them. Root hairs were found more often at temperatures lower than  $75^{\circ}$ , but this may have been simply because the cortex and therefore the root hairs persisted longer under cooler conditions.

At  $75^{\circ}$  the usual radial arrangement of protophloem and exarch xylem points was clearly apparent at 1 cm. from the root tip (fig. 5 A). In both genera the number of protoxylem groups varied from four to seven at  $75^{\circ}$ , as well as at the other temperatures employed. The extremely fine branch roots, however, usually exhibited only two or three groups each of protophloem and protoxylem.

Very fine lateral roots were most evident, however, at temperatures of  $75^{\circ}$  and higher. Note the relatively large number of fine branch roots at the higher temperatures (figs. 1-3), a situation very similar to that recorded by CONANT (1) for tobacco. The fine branch roots of apple and peach had a single layer of epidermal cells, cortical tissue only a few cells thick in cross section, an endodermis with large casparian strips, and a pericycle that showed little indication of cell division although there was some suberization in that region at  $75^{\circ}$ , just preceding death of the cortex. Shortly after emerging through the cortex of the larger "primary" root, the central cylinder of the fine laterals became lignified even to the center of the stele. There was rarely any sign of a cambium or secondary tissue. At lower temperatures there were fewer fine branch roots and most of them showed secondary thickening and consequent increase in diameter. In all other respects also they seemed similar in internal structure to "primary" roots at the same temperature.

At a point about 5 cm. back from the root tip (fig. 5 B) the "primary" roots at  $75^{\circ}$  usually exhibited externally a cortex that was more or less brown and often apparently dead. Invariably the cortical cells had matured as compared with those at 1 cm. from the tip, as evidenced by change in shape and increase in size of the

cells and intercellular spaces. In both peach and apple the cortex was clearly a juvenile structure, although, as will be shown later, it persisted at lower temperatures for the entire period of these experiments.

Concomitantly with approaching maturity of the cortex the endodermis increased in number of cells. The division of endodermal cells and newly developing casparian strips are shown in figure 5 *B*. Apparently the initiation of the cork cambium also took place at about the same time, as there was some indication of cell division in the region of the pericycle.

Cambial activity was likewise evident at 5 cm. from the root tip, although that the cambium had not been active long is clear from the rather small amount of secondary xylem and phloem which was present at this stage. Some of the growth of the central cylinder was due to cell enlargement. Especially in the center of the stele the cells were considerably larger than those at 1 cm. from the tip (fig. 5 *A, B*), but they were still rather angular, thin walled, and closely packed.

At 11 cm. from the root tip the cortex was invariably dead at 75°. Often it was completely sloughed off, although it is shown as still attached in figure 5 *C*. The torn endodermis which, at this distance from the root tip had failed to accommodate itself to the rapid growth of the central cylinder, was sometimes evident, pressed against the remains of the inner cortical tissue. The stele, however, was surrounded by an active cork cambium that produced externally an abundance of cells which suberized rapidly at 75°. Lenticels were also present at this distance from the tip. A portion of one is visible on the righthand side of figure 5 *C*. At this temperature and higher, lenticels were more numerous and larger than at lower temperatures.

Proceeding inward from the cork cambium and the more or less mature parenchymatous cells and fibers produced by it, there could occasionally be distinguished remnants of the crushed primary phloem, located of course between the fan-shaped medullary rays (fig. 5 *C*). The active cambium produced externally an abundance of secondary phloem and ray tissue far exceeding in amount that which was present at 5 cm. from the tip. Conspicuous large bundles

of strongly developed fibers were present in the region of the secondary phloem and pericycle. They undoubtedly contributed to the remarkable mechanical toughness and flexibility of these roots as already described. Also associated with the mechanical toughness and general lack of succulence, there was extensive growth of secondary xylem which was thick walled and lignified except for the xylem rays. As usual the primary rays proceeded outward from the protoxylem points; during the period of these experiments there appeared no secondary rays. In the older portion of the roots, the center of the stele, in contrast with the parenchymatous nature at a point 5 cm. from the tip, was made up of cells that had become much thickened and lignified (fig. 5 C).

#### EFFECTS OF TEMPERATURE ON ANATOMICAL STRUCTURE OF APPLE AND PEACH ROOTS

The anatomical structure of the roots at 75° has been recorded and may be conveniently employed as a basis for comparison with the corresponding histological development at higher and lower temperatures. Because under like conditions the structural development of apple and peach was essentially the same, it will be unnecessary to discuss the two genera separately. The trees of the cultures lacking an external nitrogen supply were not seriously deficient in nitrogen, as they were initially fairly high in organic nitrogenous material (table VI). The general characteristics referred to in the following discussion apply to both plus-NO<sub>3</sub> and minus-N cultures. Certain modifications effected by added nitrate will be described elsewhere.

It has already been pointed out that at 65° and lower practically the entire current root system remained white and relatively succulent for the duration of these experiments, whereas at higher temperatures the cortex rapidly turned brown and the central cylinder tough and woody. The associated anatomical situation is indicated in figures 6-9. They were made from peach roots of the complete nutrient series which were harvested on April 4.

The rate at which tissues matured was hastened by increase in temperature. At 1 cm. from the tip at 55° there was but slight differentiation of the central cylinder, the endodermis was not yet dis-

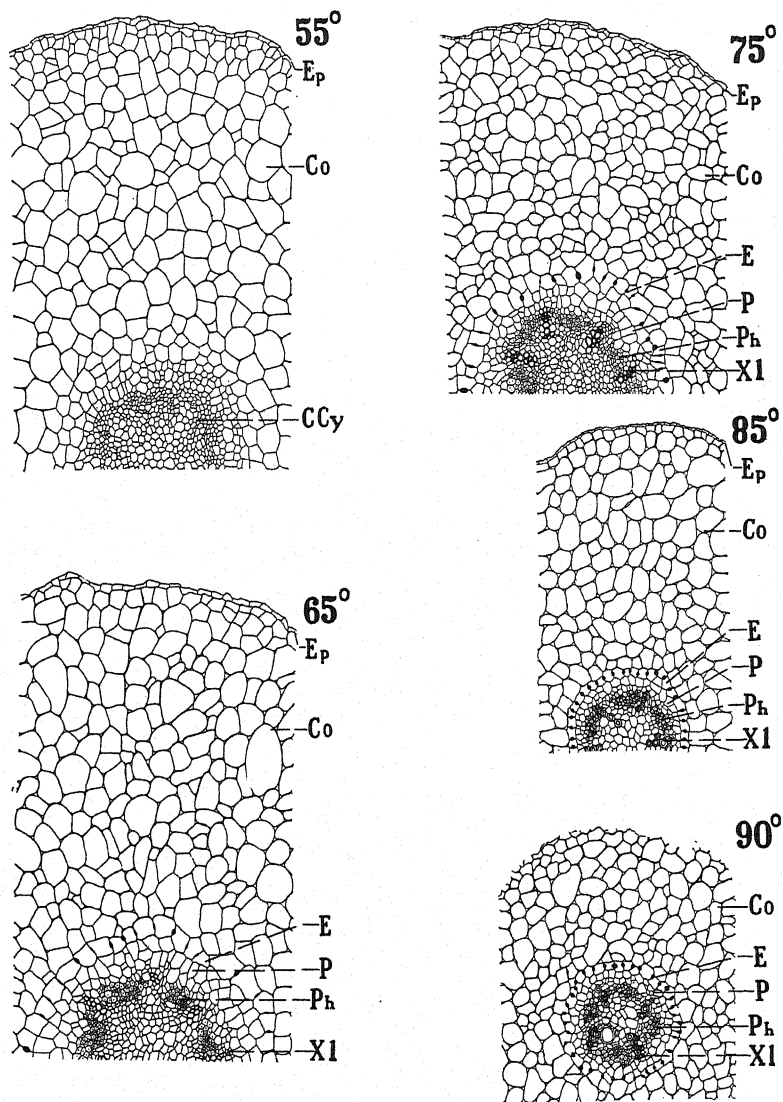


FIG. 6.—Transverse sections of peach roots 1 cm. from root tip. From low to high temperatures note decrease in number of cells and greater degree of differentiation of stele. Cortex practically dead at 85° and 90° F.

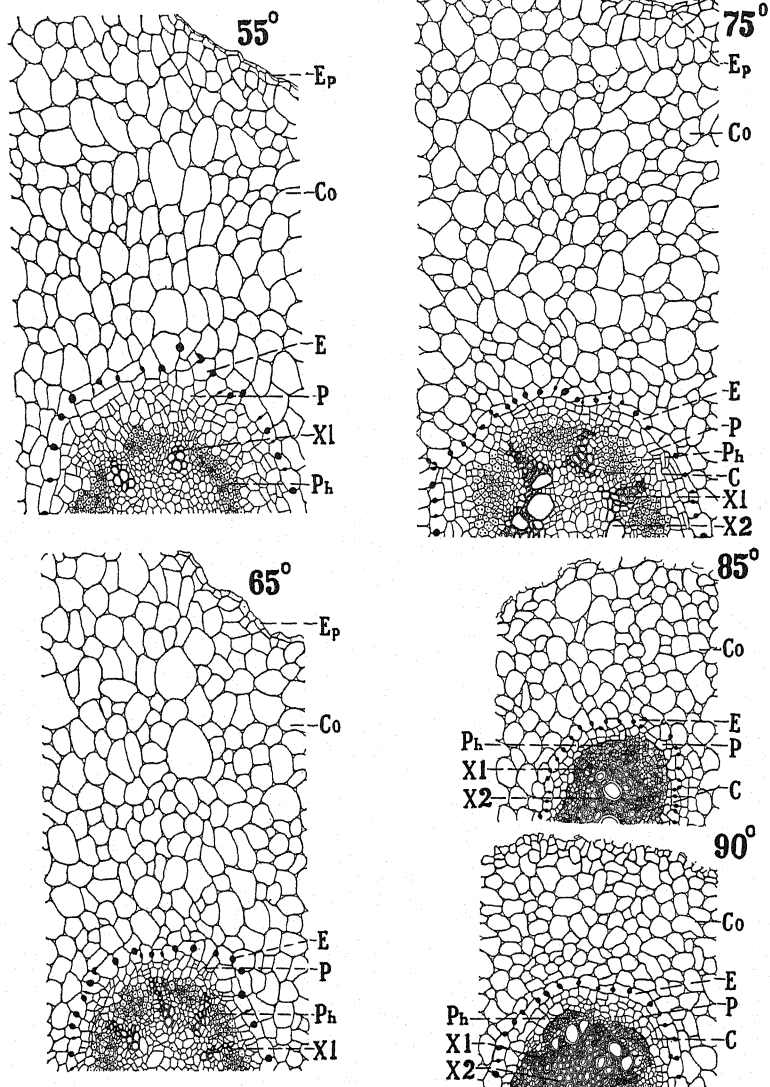


FIG. 7.—Transverse sections of peach roots 5 cm. from root tip. From low to high temperatures note greater degree of differentiation, particularly lignification of xylem, crushed primary phloem at 85° and 90°, and limited stelar cambium. Little secondary tissue below 75°. Outer cortical cells dead at 75° and entire cortex dead at 85° and 90°.

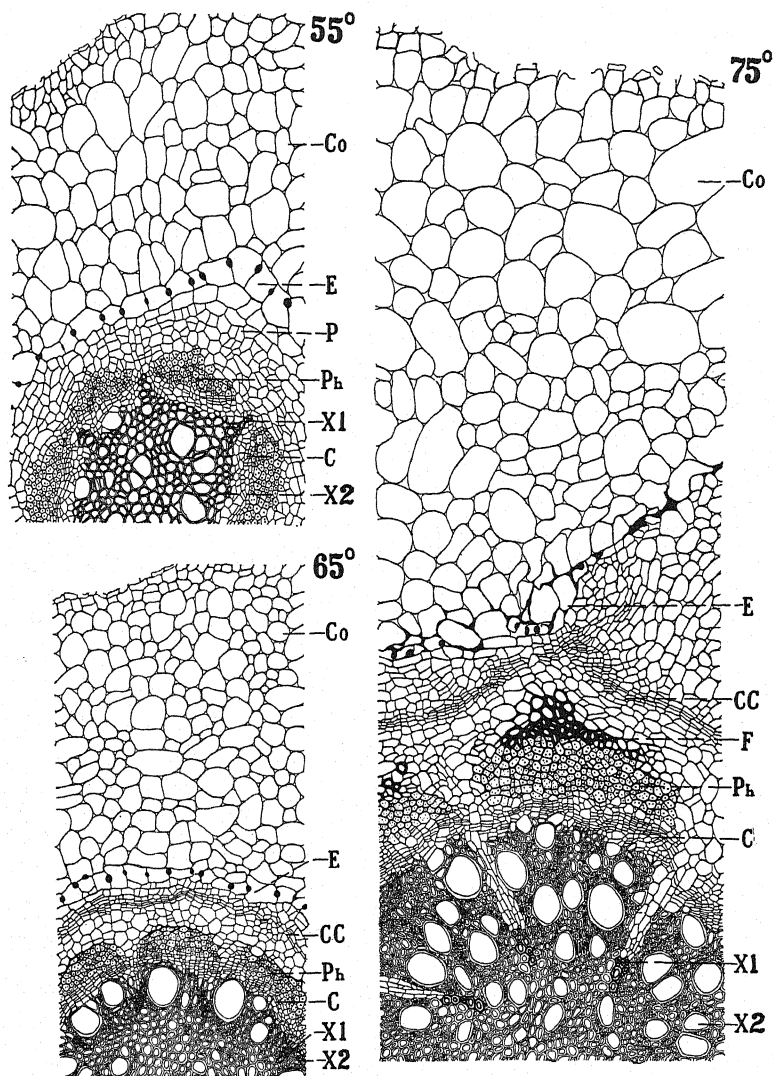


FIG. 8.—Transverse sections of peach roots 11 cm. from root tip. Cortex alive at 55° and 65° and dead at 75° (*cf.* figs. 1, 2). Note cell division in pericycle region at 55° and 65° but no suberization or fibers in contrast to well developed periderm and fibers at 75°. Primary phloem still intact at 55°, crushed at 65° and 75°. From low to high temperature note increase in amount of heavily lignified secondary xylem but presence of well developed stelar cambium at each temperature.

tinct, and the cells of the entire cross section were angular and closely packed (fig. 6). With a temperature  $10^{\circ}$  higher but at exactly the same distance from the root tip cortical cells were more mature, in that they were rounder, although still rather closely arranged. The endodermis was now distinct although the casparian strips had not reached their maximum size. In addition the central cylinder exhibited the early stages of development of the usual radial pattern of primary phloem and xylem, the latter scarcely at all lignified.

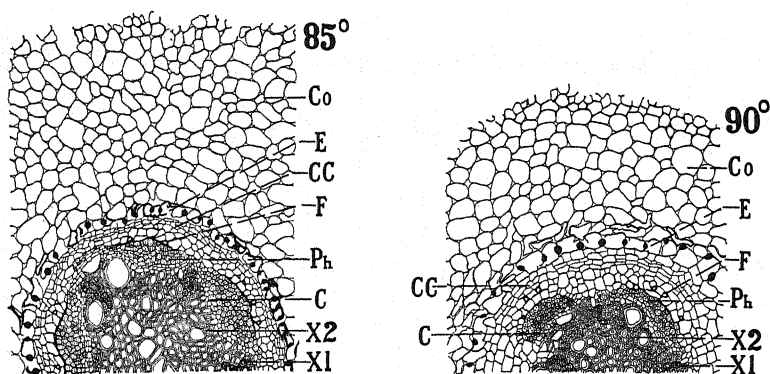


FIG. 9.—Transverse sections of peach roots 11 cm. from root tip. At both  $85^{\circ}$  and  $90^{\circ}$  cortex dead, pericyclic division limited, and periderm frequently non-continuous although cells produced externally suberized. Note at both temperatures occasional pericycle fibers, crushed primary phloem, relatively small amount of secondary phloem, and strongly lignified xylem. Amount of tissue in stelar cambium region is extremely limited, especially at  $90^{\circ}$ .

As already recorded, at  $75^{\circ}$  the protoxylem was distinctly lignified and in all other respects the roots were more advanced in maturity than at  $65^{\circ}$ . This condition was even more accentuated at  $85^{\circ}$ , and together with marked lignification of the protoxylem there were fewer cells in the potential cambium region and in the stele as a whole (fig. 6). The endodermis possessed casparian strips which at 1 cm. from the root tip were thicker than those at  $75^{\circ}$ . The cells of the cortex, although relatively small, appeared more mature, as they were much rounded, thereby resulting in large intercellular spaces.

At  $90^{\circ}$ , the highest temperature at which new current roots were produced, the total number of cells in cross section was even fewer than at  $85^{\circ}$ ; and although the sections under consideration were

taken at only 1 cm. from the root tip, the cortex was not only mature but often brown and apparently dead. The endodermis showed much the same condition as at 85°, but the central cylinder, although of relatively small diameter, had advanced even further in maturity for there was lignification of both protoxylem and metaxylem, leaving in the center of the stele only a few cells which were still parenchymatous, but much rounded (fig. 6).

The preceding observations, made on cross sections taken at 1 cm. from the tip of the roots, showed clearly that with increase in temperature there was increase in rate of maturity and differentiation of primary tissues. Further, there was indication that the smaller diameter of the roots at temperatures above 75° (fig. 6) was due, in general, to fewer primary cells rather than to conspicuously smaller cells. An exception to this generality occurred at 85° and 90°, however, where the cortical cells usually died before they apparently reached their maximum potential size.

Roots of the respective series were also examined at 5 cm. from the root tip (fig. 7). At 55° there was comparatively little secondary but much primary xylem and phloem. The central cylinder as a whole consisted of relatively immature, closely packed, thin walled, angular cells. Even the small protoxylem groups were but little lignified. In the pericycle region, just inside the now conspicuous and actively dividing endodermis, there was invariably a band of tissue four or five cells wide in cross section. As shown in figure 7, these cells were angular, thin walled, and in an active state of cell division. This is in contrast to the described development at 75° of a typical cork cambium in this region. Mention should be made of the cortex, the cells of which had become rounder than at 1 cm. from the root tip, but which, like the stele, were nevertheless far from exhibiting the typical characteristics of a mature tissue. This and other observations that follow will be further discussed in connection with the chemical composition of the roots of the respective series.

At 65° the cortex showed no sign of death, but presented much the same situation as at 55°. The stele, however, was much less active in the pericycle region. It will be shown later that at relatively mature stages it exhibited a more conventional mode of pericyclic cell division than occurred at cooler temperatures. Comparatively



little, if any, secondary tissue was apparent at 5 cm. from the root tip at 65°, but the protoxylem was much more lignified than at 55°.

Unlike that of lower temperatures, the cortex at 75° was usually brown if not for the most part actually dead, except for the endodermal cells many of which were dividing. The central cylinder showed the typical initiation of a cork cambium. There was also considerable secondary phloem and xylem, the latter becoming lignified about as rapidly as produced by the cambium. Nevertheless the central portion of the cylinder still consisted of closely packed parenchymatous cells.

This is in striking contrast to the complete lignification of comparable tissue at 85° and 90° (fig. 7). At both these temperatures there was present a cambium, but in cross section it was but one or two cells wide, being bordered on the inside by thick walled xylem and on the outside by apparently mature phloem cells. In the pericycle region there was but slight indication of cell division and none at all in the endodermis. Obviously these general results support the statements already made, that with increase in temperature there was increase in rate of maturity of primary tissues.

It is proposed to describe in the following pages the development of secondary tissue of peach and apple as it occurred at the respective sand temperatures. Sections were made at exactly 11 cm. from the root tip in each case and the results are shown in figures 8 and 9.

At 55° the cortex appeared white and under the microscope looked much the same as at 5 cm. from the tip. The endodermal cells continued to divide, but perhaps unprecedented was the conspicuous band of active cells in the pericycle region as shown in figure 8. They did not show the typical organization of a cork cambium as at 75°, but seemed rather to lack regularity in their mode of division. Further they showed not the slightest sign of suberizing. They were angular and closely packed. There were no fibers in pericycle or phloem such as those occurring at 75°, and it is apparent that the remainder of the stele also was slow in maturing, for the scalloped outline of the primary xylem was still evident although the cells of the metaxylem had enlarged and become lignified. There was a wide band of active tissue in the cambium region, but it was obviously late in forming and the fact of its width indicated slow

differentiation. Consequently there was present much less secondary phloem and xylem than at higher temperatures. As already pointed out, there was produced at 55° a surprisingly large amount of primary phloem and xylem. The cells, particularly of the metaxylem, enlarged greatly before finally becoming lignified (fig. 8). These roots have already been characterized as succulent and lacking in mechanical toughness. That they were not otherwise is clear from the anatomical development just described.

At 45° and 50° the roots appeared so late that comparable material was not available for sectioning at 11 cm. from the tip. Growth proceeded far enough, however, at 50° to make it apparent that the tissues would have eventually presented much the same situation as that at 55°. The roots at 60° exhibited, as might be expected, a condition intermediate between the higher and lower temperatures.

The cortex of the roots at 65° at 11 cm. from the tip usually appeared white, although the epidermis was sometimes broken (fig. 8). In very few instances was the cortex brown or sloughed off as at 75°. Observations of the cell contents, to be described elsewhere, also confirm the view that even at 11 cm. from the tip the cortex was far from an inert tissue. The endodermis too showed indications of recent cell division for there were evident partially thickened casparian strips. In the pericycle region also there was a well defined layer of actively dividing cells, designated as cork cambium in figure 8. Nevertheless during the period of these experiments none of the cells produced by it became suberized. The stelar cambium was active and there was derived from it a considerable amount of secondary phloem and xylem; far less, however, than at 75°. Fibers of the phloem and pericycle region were essentially lacking.

The comparative immaturity of the central cylinder at all the lower temperatures and the persistence of the juvenile cortex are in contrast to the responses occurring at 75° (fig. 8). There the cortex was completely dead if not actually sloughed off, but encircling the stele was a strongly developed cork cambium which produced external cells that suberized rapidly, whereas no suberization was observed at lower temperatures for the duration of these experiments. CONANT (1) reports that in tobacco roots also, higher temperatures

avored early development of cork cambium and rapid suberization, and that there was thereby furnished an effective mechanical barrier against penetration of the fungus *Thielavia basicola*.

At 75° there occurred the greatest development of secondary tissues, including the heavy walled fibers of the phloem and pericycle region and the strongly lignified xylem. The relatively large total mass of tissues produced per "primary" root at this temperature is indicated in figure 8. It should be emphasized, however, that although this total per root was greatest at 75°, the cortex was usually dead even to within 5 cm. of the root tips; and further, that much of the stele consisted of relatively inert or at least optically empty fibers of phloem and pericycle and lignified xylem elements. It will later be seen that this fact has apparent significance in relation to metabolic responses. This relatively large mass of dead cortex, for the most part devoid of cell contents, may also help to explain why the total root system weighed more at 65° than at the higher temperature (table II). That there was also a greater number of large roots in the 65° group has been indicated (figs. 1-3).

The highest soil temperature employed by CONANT (1) was 85°, and under the conditions of his experiments it resulted in strong cork formation in tobacco roots. In apple and peach there was only very slight sign of cell division in the pericycle at 85° and 90° (fig. 9). The outer cells of the pericycle region became suberized, although not infrequently the cortex sloughed off before this occurred, especially at 90° where an occasional fibrous root died of the plus-NO<sub>3</sub> series of apple and peach. At both temperatures a few pericycle fibers developed. It is worthy of note, however, that when shifted from 60° to 95°, the already active pericycle became strongly suberized and pericycle fibers appeared in four or five days. At the same time the wide band of cells in the cambium region rapidly differentiated and, as will be explained later, changed greatly as to cell contents, after which no cell division in the cambium region was noticeable. There was differentiation of xylem and phloem practically down to the embryonic tip, which meant of course that there was little if any growth in length.

The lack of succulence of the roots at 85° and 90° is readily understood upon even casual examination of figure 9. The cortex was dead

in both lots, the condition of the pericycle has been described, and the cambium region was only one or two cells wide in cross section at  $85^{\circ}$  while at the higher temperature there was even less. In the former case, although the development of secondary phloem was not extensive, it was sufficient to crush and compress somewhat the primary phloem which appears in figure 9 as a heavy black line between the pericycle and secondary phloem. At  $90^{\circ}$  much of the primary phloem was still intact, as there was even less secondary development. Secondary xylem too was very limited in amount at  $85^{\circ}$  and  $90^{\circ}$ , but there were more and somewhat larger vessels at the lower than at the higher temperature, in both cases strongly lignified.

The general effects on anatomy of roots as caused by a shift from  $60^{\circ}$  to  $95^{\circ}$  have been mentioned. A shift from  $60^{\circ}$  to  $45^{\circ}$  caused a marked decrease in rate of growth, although the roots continued to increase considerably in length and diameter. The increase in length was made evident by a remarkably long zone of elongation between embryonic tip and region of maturation; the diametric increase was evidenced by a band of cambium unusually wide in transverse section and by pericycle several cells in width, exhibiting much the same type of cell division and lack of suberization as that described for the series continually at  $55^{\circ}$ . While at  $45^{\circ}$  the cortex persisted, there was little noticeable lignification of newly formed xylem, and in all other respects the roots were slower in maturing than those which were allowed to remain continually at  $60^{\circ}$ .

On March 17, when some of the cultures which had been continuously at  $45^{\circ}$  were shifted to  $60^{\circ}$ , current roots were entirely lacking; but by April 4 there were newly developed roots nearly equal in volume and weight and similar in external appearance and anatomy to those produced by the trees continually at  $60^{\circ}$ .

#### EFFECTS OF TEMPERATURE ON HYDROGEN ION CONCENTRATION OF ROOT TISSUES

There has been presented evidence to show that the external appearance of the roots of the respective series was intimately associated with their anatomical character, and that both were greatly modified by comparatively small differences in temperature of the

nutrient medium. Estimations of H ion concentration leave much to be desired as they give no indication of the quality or quantity of the various substances in ionic equilibrium (10). Nevertheless, the pH values of root tissues were found to be closely associated with the growth responses already recorded.

The conventional range indicator method (27) was employed and a large number of microscopic observations were made of the roots of the respective series immediately after they were removed from the sand cultures. However, it is unnecessary to present a long list of detailed observations here. The pH values given apply to both apple and peach, since respective tissues of the two genera were not noticeably different in H ion concentration under comparable conditions. The situation is briefly summed up in the following pages.

Mature xylem elements wherever observed, whether produced at continually high or low temperature, or whether developed following a shift from one temperature to another, were invariably optically empty and only the walls reacted to indicators. The pH of insoluble walls was perhaps not determined in the usual sense of the symbol, yet the reaction to such indicators as methyl red gave a so-called pH value of the lignified walls of 4.4-4.0, possibly an indication of the strength of the acids concerned. These figures would seem worth recording, however, for they were characteristic of mature xylem of the roots under the several temperature conditions. In this connection it will be recalled that lignification proceeded slowly at temperatures below 75°, whereas at this temperature and above a relatively large percentage of the root system consisted of strongly lignified xylem elements that invariably gave the acid reaction mentioned.

Cellulose walls failed to give distinct color tests, but fibers of pericycle and phloem, which it will be remembered were present only at 75° and higher temperatures, were distinctly acid. Fibers in the process of developing gave a reaction of 4.6-4.0, but some of the mature fibers were estimated to be approximately pH 3.8.

The cell contents of primary and secondary phloem were much less acid. Recently developed phloem cells regardless of their origin contained protoplasm which had a pH value of 6.2-5.9, but protoplasm of mature cells was pH 5.2-4.8. It will be recalled that the

phloem matured rapidly at higher temperatures and there was a relatively low percentage of it as compared with the rather large proportion of phloem which was present and matured slowly at temperatures below 75° (figs. 6-9), consequently the average pH of the phloem at 75° and higher was more acid than that at lower temperatures. The content of mature cells of phloem and xylem rays was consistently pH 4.4-4.0. As usual, the cellulose walls failed to give a distinct reaction.

The real pH of the stelar cambium is perhaps impossible of estimation. At 90° and 85° there was so little tissue in the cambium region, only one or two cells wide in cross section, and the protoplasmic contents were so limited in amount, that possible color reactions were masked by reflection of color from adjacent walls of the mature xylem and bordering phloem cells. At 75°, however, there was in transverse section a wide band of tissue separating xylem and phloem (fig. 5). The cells, particularly in the middle of this cambium zone, exhibited dense protoplasmic contents and were actively dividing. Freshly cut sections placed on a slide with no coverslip gave in this region a pH value of 5.2-4.8. This occurred even though the sections had been successively and rapidly washed several times with new applications of the non-alcoholic, aqueous indicator solution employed (27). However, in the course of half an hour or less the cambium region became much less acid, approximately 6.2-6.0, and remained so for several hours. But if a coverslip was placed over the sections the cambium region and often the phloem again exhibited a more acid reaction, pH 5.2-4.8, probably owing to accumulation in these tissues of carbon dioxide while under the coverslip. A similar observation has been made by SMALL (27).

The initial comparatively acid reaction may likewise have been due to carbon dioxide, but to carbon dioxide actually present in solution in the active cells of the growing roots at 75°. MAGNESS (17) and others (27) have found that when respiration is rapid, carbon dioxide may accumulate in plant tissues in amounts sufficient to account for this pH change.<sup>3</sup> Respiration was doubtless (23) much

<sup>3</sup> THORNTON (31) exposed the living hyphae of *Sclerotinia fructicola* to an atmosphere containing 20 per cent oxygen and various concentrations of carbon dioxide ranging from 10 to 60 per cent. The several treatments all resulted in a decrease in acidity of the liv-

more rapid at 75° than at lower temperatures, and there would accordingly be present relatively much carbon dioxide.

At 65° and lower the cambium did not have an initial pH as acid as that recorded for the roots at 75°, but with no coverslip had a H ion concentration of about 6.2–6.0. This would seem to indicate that there was in the growing roots a lower concentration of carbon dioxide than at 75°, owing presumably to less rapid respiration at the lower temperatures. The application of a coverslip resulted, as at 75°, in a more acid reaction of all tissues containing protoplasm, principally the cambium, young xylem, and recently developed phloem, apparently indicating that at laboratory temperature these tissues respired rapidly. A similar response was likewise obtained shortly following a shift in cultures from 60° to 95°. As will be shown later, there was available an abundance of carbohydrates for respiration at both 60° and 65°.

At 85° and 90° the pH values of cells, exclusive of the embryonic zone of the root tips, remained unaffected by the presence or absence of a coverslip, probably because of limited evolution of carbon dioxide as there were few active cells containing protoplasm. Lack of respirable material must also have been a modifying factor and will be discussed further in connection with the composition of the roots. In the embryonic zone of the root tips there was at these temperatures an apparently high concentration of carbon dioxide; at least the active tissues of this region had an initial pH of 5.0–4.8, which changed on standing with no coverslip to 5.8–5.4. Much the same situation was observed in the meristematic tissues of the root tips at 75°, but at the lower temperatures the initial and final pH was 5.8–5.6. Again the reason would seem to be that at lower temperatures respiration was slower, and therefore little carbon dioxide accumulated. That the embryonic tissues of the root tips at low temperatures were capable of rapid respiration would seem to be indicated by the increase in acidity which occurred upon leaving sections

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ing hyphae from about pH 5.6 to about 7.2. The formation of carbonates if such occurred might account for this pH change, but carbonates were not determined and no explanation is as yet available. On the other hand effects on the protoplasm of an external supply of carbon dioxide might well be different from that of rapid respiration and resulting increase in carbon dioxide.

under a coverslip at laboratory temperature for a few minutes. Furthermore, shortly after shift of cultures from 60° to 95° there occurred a marked increase in acidity of all meristematic tissue, undoubtedly owing in large part to increased respiration and consequent greater content of carbon dioxide.

The real pH of meristematic tissue and of cambium may therefore be problematical, but it would seem to be less acid than that of xylem walls (4.4-4.0), and to approach the less acid condition of young phloem cells (6.2-5.9). Of course there was no clear line of demarcation between cambium and xylem. Especially at lower temperatures they intergraded insensibly one into the other. As the cells of the inner surface of the cambium differentiated, there was loss of cell contents from the developing xylem elements and the walls became more acid as lignification progressed. A more or less parallel situation also occurred in the embryonic zone of the root tips, where the corresponding mature primary tissues had about the same pH value as those which were derived from the cambium. In this connection it may again be noted that the highest percentage of meristematic tissue was found at the lower temperatures. The amount of tissue giving a less acid reaction, therefore, became increasingly less at higher temperatures where the more acid xylem elements and fibers of phloem and pericycle predominated.

At 75° and higher, the pericycle on its inner surface produced fibers already described and parenchymatous cells which had a pH value of 5.2-4.8. On the outer surface of the pericycle the walls of the suberized cells showed a reaction to indicators of 4.2-4.0. The actual pH of the cork cambium could not be determined on account of reflection of colors from the adjacent tissues produced by it. The pH value was probably not much, if any, less acid than 5.2, as otherwise indicators negative in reaction to adjacent tissues would have given a positive test in the cork cambium region.

The endodermis was among the first structures of the root to be clearly differentiated, and from that time until maturity the cell contents exhibited a pH value of 4.4-4.0. The casparian strips also gave a positive reaction at approximately the same H ion concentration. There was no noticeable difference in this respect between



roots of the several series, and the same values were maintained as long as the endodermis persisted.

Most of the cells in the remainder of the cortex were much less acid (5.2-4.8), although there was a considerable number of scattered cortical cells, singly or in small groups, which gave a reaction of 4.4-4.2. The contents of such cells are described later. As the cortex matured, the protoplasmic content decreased and the proportion of relatively acid cells became higher. When dead it was practically devoid of cell contents and the brownish walls gave no noticeable reaction. It will be recalled that at 65° and lower all cortical tissue persisted for the duration of these experiments, whereas at higher temperatures the cortex matured rapidly and died.

At temperatures of 75° and higher, relatively young cortex from sections made near the root tips exhibited an initial reaction of about 4.4, but on standing for a few minutes the majority of the cells became less acid. This was undoubtedly due in part to the so-called "wound carbon dioxide," but was nevertheless much more pronounced at 75° than at 65° or lower. It probably was associated with higher carbon dioxide content and more rapid respiration in the roots grown at higher temperatures.

The epidermis of washed roots apparently had a more or less permanent pH value of about 4.4-4.2, but the surface of the current roots as they existed in the sand cultures was modified by the nutrient treatment. The trees lacking nitrogen in the culture solution had a pH value at the unwashed root surfaces of about 4.4-4.2, approximately that of the minus-N nutrient solution as applied. For trees receiving nitrate the corresponding value varied from 4.8 to 6.0. The less acid condition was found where nitrate was being absorbed and assimilated most rapidly, and was undoubtedly due, at least in part, to more rapid absorption of nitrate than of basic ions by the roots under the conditions of these experiments. This point has been discussed in connection with the results of other work (19).

The observations so far recorded show that the rapidly maturing non-succulent roots at temperatures of 75° and higher lost their cortex early, and were comparatively high in proportion of strongly lignified xylem, suberized tissue of the periderm, and mechanical fibers of phloem and pericycle, all of which exhibited an acid reac-

tion. On the other hand they were low in proportion of less acid tissues, such as young phloem and cambium, and the embryonic zone of the root tips was also very limited in volume. Such meristematic tissues as were present were relatively acid, owing apparently to a high content of carbon dioxide coupled presumably with a high rate of respiration.

In contrast, the roots at lower temperatures were characteristically succulent and the cortex persisted. The stele was slow in differentiating and consisted of relatively much active tissue that was notably less acid than mature tissues, a pericycle that was extremely active but did not suberize or produce fibers, phloem that was likewise entirely lacking in mechanical elements, and cambium which was in an active state of cell division but produced on its inner surface xylem that lignified and reached its maximum degree of acidity very slowly.

All the foregoing facts on anatomy and pH of tissues have an important bearing upon the observations which are recorded in the following pages.

#### EFFECTS OF TEMPERATURE ON ABSORPTION AND ASSIMILATION OF NITRATE BY APPLE AND PEACH ROOTS

Under the conditions of these experiments, temperature did not materially limit the ability of the trees to absorb nitrate. Some of the apple and peach trees lacking nitrate were later supplied with the complete nutrient solution (table I). On the basis of nitrate appearance within the current roots of these trees no consistent difference in permeability could be detected. It was only a matter of minutes after application before the entire current root system of both apple and peach contained nitrate in abundance, regardless of the temperature of the sand in which the trees were growing.

Without exception, nitrate was limited exclusively to the current roots for the entire period of these experiments. Why nitrate was found only in these organs is not clear, but inorganic nitrogen during most of the year is commonly limited to the fibrous roots of apple (7, 19, 22, 30), peach (3, 23), and other perennial plants (20, 21). DAVIDSON and SHIVE (3) found that nitrate was present, however, in the aerial organs of peach when an alkaline reaction of the nu-

trient medium apparently prevented (3, 19, 32, 33) utilization of nitrate by the roots. STUART (28) likewise reports nitrate in the tops of young apple trees which received excessive applications of nitrate and exhibited leaf injury. ECKERSON (7) and THOMAS (30) found nitrate in the tops of apple trees but only in the buds as they were opening. ECKERSON states, "It seems safe to conclude that if there is nitrate in the soil some will be carried to the buds when they begin to swell in the spring, and for a short period the buds (and adjacent bark) take an active part in the reduction of nitrate and synthesis of amino acids." ECKERSON's work and that of others cited shows clearly that the fibrous roots are the organs chiefly concerned in the assimilation of nitrate nitrogen. Under the conditions of these experiments they were apparently the only organs of apple and peach that were engaged in this phase of nitrogen metabolism.

Whereas the temperature of the nutrient medium affected little, if at all, the ability of the roots to absorb nitrate (provided they were not already saturated to capacity with it), temperature had a very marked influence on the capacity of the roots to reduce nitrate to nitrite, ammonium, and amino acids. This process of reduction and synthesis may be conveniently included in the term *nitrate assimilation*. The relative rate at which this phase of protein synthesis takes place is apparently indicated with a fair degree of accuracy by ECKERSON's method (6), which briefly consists of taking an aqueous extract of fresh plant tissue and measuring the amount of nitrite reduced from nitrate by a given sample under specific conditions of time, pH, and temperature. The amount of nitrite formed from nitrate gives a measure of the *reducase* activity of the particular plant or organ sampled. Analyses have repeatedly shown (6, 8, 18, 22) that *reducase* activity closely parallels the synthesis in the plant of amino acids and other forms of elaborated nitrogen. *Reducase* activity therefore furnishes an index of the relative rate of nitrate assimilation.

#### *Nitrate assimilation by apple*

Aliquots of the entire current root system of apple were employed during the early part of these experiments and determinations made of *reducase* activity. When the first harvest took place during early

March, reductase activity seemed to be roughly proportional to temperature, the maximum reduction occurring at higher temperatures (fig. 10). But as the experiments progressed, at temperatures of 75° and higher, there occurred a marked decrease in ability of the roots to reduce nitrate; whereas at 65° and lower the respective curves for reductase activity dropped very little.

Such a response does not seem surprising, however, since at higher temperatures, with each later harvest, the samples of whole current roots became increasingly high in proportion of dead cortex and relatively inert xylem along with mechanical elements of phloem and pericycle. It will be recalled that at 65° and lower the cortex persisted, differentiation of lignified xylem was slow, and no fibers appeared in either phloem or pericycle. In addition, the respective tissues at lower temperature were relatively less acid, a fact probably of considerable significance. A slightly alkaline condition greatly accelerates nitrate reduction in the plant, at least in the case of tomato (5).

It therefore appeared probable that at higher temperatures reduction of nitrate occurred principally in recently developed and still active portions of the root system which were comparatively less acid. For a comparison of nitrate reduction at different temperatures, samples were accordingly taken in the vicinity of the root tips, care being observed to avoid any possibility of desiccation. The length of the so-called root tips varied from 3 to 5 cm. The chief object was not to secure samples of any particular length, but to obtain recently developed tissue that still contained a high proportion of cells with comparatively dense protoplasmic contents.

The aliquots of entire current roots of the early harvests were approximately in this category as they had been growing for but a short period. The reductase determinations on these, together with those for the root tips harvested on subsequent dates, gave the results for reductase shown in figure 10. The curves vary somewhat. They are not straight lines but they show beyond any reasonable doubt that the rate of nitrate reduction increased with increase in temperature. They show too that it varied little from week to week under these conditions where there were present cells which contained abundant protoplasm. Samples for reductase determinations were likewise

taken from the proximal or oldest end of roots of the respective series. The results are not reported in detail as they simply corroborate those already recorded. At  $75^{\circ}$  and higher, where the cortex was completely dead and the central cylinder in large part made up of apparently inert tissues, such as lignified xylem and fibers of phloem and pericycle, there was practically no nitrate reduction, a finding seemingly in accord with the work of ECKERSON (7) who reports little or no reducease in the larger fibrous roots of apple. In contrast, at  $65^{\circ}$  and lower, where the cortex was still alive and the stele much less mature (figs. 6-9), reducease activity at any given temperature was nearly as high at the proximal as at the distal end of the root.

Reducease determinations were also made before and after shifting the apple trees from one temperature to another. The results are expressed in milligrams of nitrogen as nitrite per gram of fresh tissue. On March 17, the roots at  $60^{\circ}$  had a value of 0.14 mg. of nitrite nitrogen, but at the beginning of the third day after shifting to  $95^{\circ}$  the corresponding figure was 0.20, two days later 0.07, and by the last of March there was practically no nitrate reduction. Obviously a temperature even as high as  $95^{\circ}$  increased reducease activity but only for a comparatively brief period, while protoplasm was still relatively abundant. It will be recalled that shortly following the shift from  $65^{\circ}$  to  $95^{\circ}$  there was rapid differentiation of tissues, lignification of xylem, and death of the cortex. Essentially the same response occurred in another set of experiments with apple (22) and likewise with tomato (18) when shifted from a relatively low temperature to  $95^{\circ}$ .

On the other hand, some of the apple trees of the group at  $60^{\circ}$  were shifted on March 17 to a sand temperature of  $45^{\circ}$ . Just before shifting and at intervals until April 4, reducease tests were made. In chronological order the results were: on March 17, 0.14; on following dates 0.12, 0.10, 0.05, and 0.13 mg. of nitrite nitrogen per gram of fresh weight of the current roots. There was thus apparently maintained a comparatively high plane of reducease activity in these roots, which matured little if any following the shift to low temperature, and which before being transferred from  $60^{\circ}$  to  $45^{\circ}$  were fairly high in nitrate reducing ability.

Previous work (22) with the same variety of apple (Stayman)

showed that there was apparently synthesis of amino acids by the current roots during a period in which the trees were held at 45°. It may be remarked, however, that the organic products of nitrate reduction largely remained in the roots in case of Stayman, whereas Baldwin translocated the newly synthesized products of nitrate reduction to the tops. Both roots and aerial organs were subjected to the same temperature.

It has been pointed out that the rate of nitrate reduction increased with rise in temperature, even to 95°, the highest employed in these experiments. This was true, however, only when relatively young active tissues were employed for testing. Low temperature favored the production of roots which were proportionately high in such tissues, but at high temperatures the cortex died early and in the stele the predominating tissues were woody, lacking in protoplasm and in ability to reduce nitrate (figs. 6-9).

It seemed logical, therefore, to find that the current root system at the intermediate temperature of 65° was highest in absolute reduction of nitrate as computed on the arbitrary basis of nitrite produced *in vitro* (fig. 11). It is true that the greatest volume and weight of roots were produced at this temperature, and that none of the tissues included in the yield recorded were dead, as was much of the cortex at higher temperatures. But it is not without significance that the roots were comparatively high in proportion of active tissues and low in relatively inert elements, such as mechanical fibers, lignified xylem, and strongly suberized periderm.

In this connection it may be noted that the curves for fresh weight of current tops produced at the respective sand temperatures (fig. 11) closely paralleled the curves for absolute reducase activity per current root system. A little consideration makes it appear doubtful, however, whether a continuous temperature of 65° would be desirable, for the root systems grown at that temperature were lacking in mechanical strength as compared with those at 75°.

#### *Nitrate assimilation by peach*

Before considering the chemical composition of the current roots, it should be stated that negative results were obtained in all attempts to demonstrate reducase activity in peach. The extracts of

current roots, old roots, old stem, and current tops were adjusted to various pH values in addition to the usual one of 7.2. Cyano-genetic material was also removed by hydrolysis with emulsin with accompanying aspiration into receiving flasks containing alkali (4), following which the cyanide-free extracts were tested in the usual manner for reducase activity, but no nitrate reduction occurred.

These tests were conducted over a period of only two weeks, using as material Elberta peach trees that had been grown under the greenhouse conditions of December and January in sand culture at a temperature of 60°–70°. The current roots were in external appearance, anatomy, and composition much like those of the groups grown continually at 65°. Occasional reducase tests were made on peach trees during the course of the experiments, but the extracts failed to give evidence of nitrate reduction. The lack of positive results may mean little, however, as the trials did not cover a sufficient period of time in the growth cycle of peach. Apple may exhibit little or no reducase activity for a considerable length of time as grown under commercial conditions in the orchard (7). In case of the plant material available, it therefore seemed more expedient in these particular experiments to study nitrate assimilation in peach by micro-chemical observation, and by measurements of the assimilated or nitrate-free nitrogen present in the current roots before and after addition of nitrate. The analytical data so secured are given in tables V and VI. These data and the current growth of tops obtained (fig. 4) made it appear that the general course of nitrate reduction in peach was essentially the same as in apple under comparable conditions.

Also, strong nitrite reactions were obtained only in the current roots of both genera. This product of nitrate reduction appeared in about 24 to 36 hours at 60° and 65°, and in from 12 to 18 hours at 75°. At higher and lower temperatures nitrite tests gave negative results, possibly because present in extremely small amount at any one time, or nitrite may have been present for a brief period during which time the plant material was not examined. Even at the intermediate temperature of 65°, nitrite was, as usual (5, 18), present for a period of only a few hours, shortly following the initial applica-

tion of nitrate. Nitrite is not generally found in quantity in plants continually supplied with an external source of readily available nitrate.

EFFECTS OF TEMPERATURE ON CHEMICAL COMPOSITION  
OF APPLE AND PEACH ROOTS

*Nitrogenous material in root systems of apple*

The apple trees were continually supplied with the complete or plus- $\text{NO}_3$  nutrient solution. The results of analyses of the current roots are given in table III. At the extremes of temperature employed there was insufficient tissue for macroanalysis.

TABLE III  
STAYMAN APPLE TREES  
DRY MATTER, CARBOHYDRATES, NITRATE, AND NITRATE-FREE NITROGEN  
IN CURRENT FIBROUS ROOTS ON APRIL 4, EXPRESSED AS  
PERCENTAGE OF ASH-FREE GREEN MATTER

SAND TEMPERATURE (°F.)	DRY MATTER	REDUCING SUGARS	SUCROSE	STARCH AND DEXTRIN	NITRATE- FREE NITROGEN	NITRATE NITROGEN
50*	6.8	0.13	0.08	0.50	0.41	0.012
55	7.3	0.09	0.11	0.39	0.45	0.014
60	7.0	0.10	0.06	0.71	0.30	0.010
65	9.0	0.11	0.07	1.04	0.27	0.009
75	12.0	0.03	0.00	0.83	0.13	0.011
85	15.2	0.01	0.00	0.20	0.09	0.016
60-45†	7.5	0.17	0.12	1.74	0.26	0.017
60-95†	11.7	0.03	0.00	0.42	0.14	0.013

\* Amount of current root growth was inadequate for macro-analysis at 45°, 90°, and 95°.

† Shifted March 17.

It is obvious that there was an abundance of nitrate in the current roots of each group of trees, and that the difference in concentration of nitrate under the several temperatures was perhaps too small to be significant. It may be noted, however, that at 65°, where reducase activity was highest (figs. 10, 11), the nitrate content was lowest and it was somewhat higher at 85° and 50° where the assimilation of nitrate was less vigorous. Likewise, when some of the trees at 60° were shifted to 45° and 95° respectively, the percentage of nitrate nitrogen increased materially. It is not uncommon for nitrate to ac-



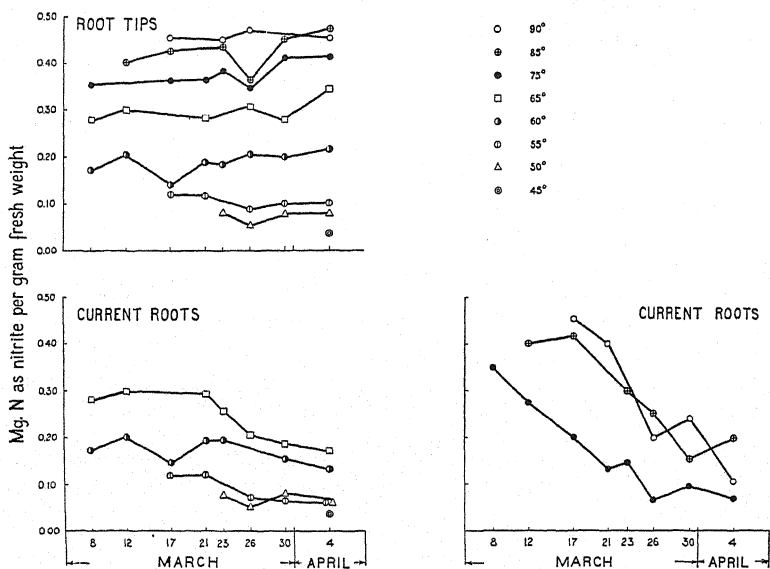


FIG. 10.—Reduction of nitrate by extracts from tips of apple roots and by extracts from aliquots of complete current root system. Roots grown at temperatures indicated, the tops at about 65° F.

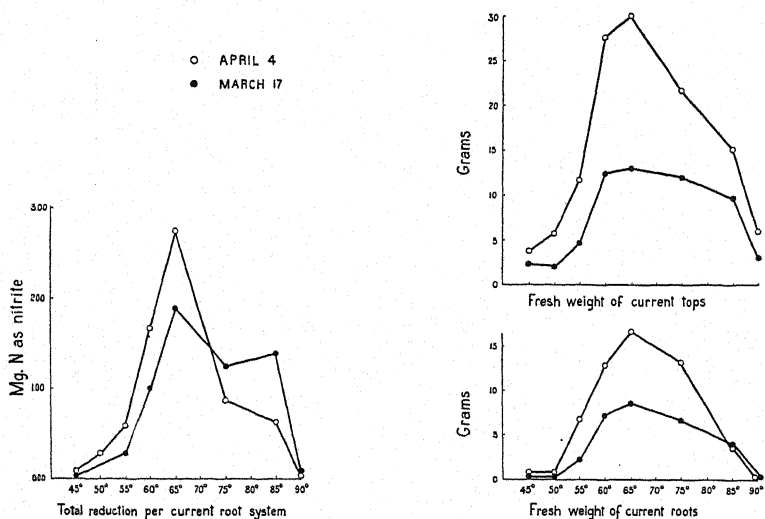


FIG. 11.—By computation, absolute reduction of nitrate by entire current root system of apple and fresh weight yield of current roots and tops. The aerial organs were grown at about 65°, the roots at temperatures indicated.

cumulate in plants when there is little utilization of it in protein synthesis.

The column of percentage figures under the heading Nitrate-free nitrogen (table III) furnishes an index to the concentration of organic nitrogen, including such compounds of elaborated nitrogen as asparagine, amino acids, and proteins. It is evident that the low temperatures of these experiments were associated with the greater concentrations and high temperatures with the lower concentrations of organic nitrogen in the current roots. Even at 65°, although reductase activity was greatest here (fig. 10), there was much less assimilated nitrogen, on a percentage basis, than at lower temperatures.

In view of the results on reductase activity, these data might seem unreasonable if there was not available information concerning the anatomical structure of the root systems concerned. Meristematic and relatively undifferentiated tissues are notably high in proteins and they were the predominating type of tissue at lower temperatures. It will be recalled that differentiation was slow, that the cortex persisted, and that much of the stele remained parenchymatous for a considerable time and lost protoplasm (incidentally proteinaceous material) very slowly at temperatures lower than 65°. At 75° and above the reverse situation occurred; there the relatively high proportion of dead cortex, necessarily included with the living tissue for chemical analysis, was essentially devoid of all cell contents and the stele consisted in large part of relatively inert elements such as suberized periderm, mechanical fibers of pericycle and phloem, and strongly lignified, optically empty xylem. All these tissues were presumably low, if not entirely lacking, in organic nitrogen and gave no positive tests for it.

In these experiments the high percentage of organic nitrogen was intimately associated with the comparatively immature condition of the roots. This should not be confused with the results of earlier work (22) in which accumulation of organic nitrogen occurred in root systems of Stayman because at 45° the newly synthesized amino acids were apparently not freely translocated to the tops. During the brief period that the trees were held at that temperature the initially present root systems there concerned were relatively

more mature in anatomical character and therefore lower in proportion of meristematic tissues. Nitrogen was there stored in large part as amino acids and asparagine. In the present experiments much of the organic nitrogen of the roots at low temperature was in the form of complex proteins of the cambium, pericycle, phloem, and of the extensive embryonic zone of the root tips.

The shift from a sand temperature of  $60^{\circ}$  to  $45^{\circ}$  in these experiments had no appreciable effect upon the percentage of organic nitrogen in the current roots (table III). The tops were grown at an air temperature of about  $65^{\circ}$ , however, and there may well have been translocation of organic nitrogen to the actively growing leaves and stem. In the previous experiments just cited (22) there was practically no growth of tops when the air as well as the sand was held at  $45^{\circ}$ .

Some of the trees of the  $60^{\circ}$  group were likewise shifted to a sand temperature of  $95^{\circ}$ . It will be recalled that shortly following the transfer there occurred differentiation of the stele and death of all cortical tissue nearly to the root tip. This marked decrease in amount of embryonic and relatively immature tissue with increase in proportion of heavily lignified xylem and mechanical elements of the periphery of the stele resulted in the marked drop in concentration of organic nitrogen shown in table V.

Concomitantly with differentiation of tissues and decrease in amount of protoplasm, complex proteins decreased, but there was no noticeable accumulation of proteolytic material. The products of protein hydrolysis were probably rather rapidly translocated to the tops; at least the terminal leaves temporarily became relatively soft and dark green and the developing internodes comparatively long and spindling. Microscopic observations also showed that the tips of the stems and especially the petioles were high in concentration of soluble organic nitrogen.

As already mentioned, no nitrate was found in the old roots, but it is significant that the percentage of organic nitrogen in these initially present structures (table IV) corresponded closely in trend to the curve for absolute reduction of nitrate as shown in figure 11. The detailed analyses of the tops are not reported, but a similar

situation was found in the old stems, current stems, and leaves. The growth of tops (fig. 4) and the organic nitrogen content of aerial organs and old roots (table IV) thus practically paralleled the curve for absolute reducase activity of the respective current root systems (fig. 11), tending to show that the reducase determinations furnished a fairly accurate index of effects of temperature on nitrate assimilation as described in preceding pages.

TABLE IV  
STAYMAN APPLE TREES  
DRY MATTER, CARBOHYDRATES, AND NITRATE-FREE NITROGEN IN OLD ROOTS  
ON APRIL 4, EXPRESSED AS PERCENTAGE OF GREEN MATTER

SAND TEMPERATURE (°F.)	DRY MATTER	REDUCING SUGARS	SUCROSE	STARCH AND DEXTRIN	NITRATE- FREE NITROGEN*
45.....	58.00	1.59	0.71	5.55	0.051
50.....	50.00	1.91	0.80	4.00	0.054
55.....	45.20	0.94	0.33	4.17	0.072
60.....	43.33	0.70	0.44	4.76	0.081
65.....	43.33	0.83	0.38	4.30	0.070
75.....	38.86	0.54	0.25	3.12	0.068
85.....	34.61	0.71	0.38	2.00	0.050
90.....	42.70	0.83	0.32	4.11	0.055
95.....	43.00	0.50	0.27	4.20	0.060
(Initial Feb. 1)	(57.80)	(1.82)	(0.77)	(6.07)	(0.052)

\* No nitrate was found in the old roots of any of the trees at any time.

#### *Nitrogenous material in root systems of peach*

All of the peach trees were grown with no external supply of nitrate until March 17. At that time the foliage was fairly dark green and there was little, if any, evidence of nitrogen deficiency as the old roots and old stem initially contained an abundance of nitrogen (table VI). As already stated, the volume of current top growth followed closely in trend the production of roots at the respective sand temperatures. Nitrate applied on the date mentioned was immediately absorbed in liberal amount in all cases and was limited to the current roots. The percentage of nitrate nitrogen in these organs at the time of final harvest on April 4 is shown in table V. The variations in concentration of nitrate at the several temperatures were

small, but at 85°, 90°, and in the set shifted from 60° to 95°, nitrate is relatively high, possibly because of limited synthesis of organic nitrogen. At least the addition of nitrate resulted in practically no increase in organic nitrogen in the new or in the old roots (tables V and VI) of the cultures concerned.

The concentration of nitrate-free nitrogen in the respective current root systems on March 17 is not low, even though there was available no external supply of nitrogen. The initially present old roots and old stems must obviously have furnished the entire amount of nitrogen which contributed to the growth of new roots and aerial organs. It was undoubtedly made available mainly through proteolytic changes and subsequent translocation to current roots and tops.

Some of the peach trees which received the nutrient solution lacking nitrogen were harvested for analysis on March 17 (table V). As in apple, the concentration of nitrate-free nitrogen in the current roots was found to be highest at low temperatures. This was not because there was more rapid proteolysis or greater reutilization of stored proteins under cooler conditions. The amount of organic nitrogen in the current root systems at low temperatures was obviously not great as the amount of root growth was relatively little (table II; figs. 1, 2). The high percentage of organic nitrogen was associated with the high proportion of relatively meristematic tissues present at 65° and lower. Similar responses occurred in apple and have already been discussed in considerable detail.

Essentially the same situation was found when the remainder of the trees which lacked nitrate were harvested for analysis on April 4 (table V). The only result possibly requiring comment is the relatively high percentage of organic nitrogen in the current roots following the shift from 60° to 95°. This probably does not represent an increase in absolute amount of organic nitrogen so much as an increase in percentage due to loss in carbohydrates (table V). Nevertheless, with the marked drop in dry matter and carbohydrates which occurred in the old roots following the shift (table VI), there may well have been hydrolysis of stored proteins in the old roots and subsequent translocation of the proteolytic products to the current roots. At least with decrease in dry matter the old roots, instead of



TABLE VI  
ELBERTA PEACH TREES  
DRY MATTER, CARBOHYDRATES, AND NITRATE-FREE NITROGEN IN OLD ROOTS EXPRESSED AS PERCENTAGE  
OF GREEN MATTER

SAND TEMPERATURE (°F.)	MARCH 17					APRIL 4				
	MINUS-N					MINUS-N				
	DRY MATTER	REDUC- ING SUGARS	SU- CROSE	STARCH AND DEX- TRIN	NI- TRATE- FREE NITRO- GEN†	DRY MATTER	RE- DUCING SUGARS	SU- CROSE	STARCH AND DEX- TRIN	NI- TRATE- FREE NITRO- GEN†
45.....	49.5	1.2	0.9	5.0	0.041	49.0	1.5	0.5	5.3	0.040
50.....	50.0	1.3	0.8	4.8	0.050	49.0	1.7	0.8	5.2	0.040
55.....	48.2	1.1	1.0	5.2	0.042	43.0	1.5	0.8	5.7	0.042
60.....	47.0	0.9	0.6	4.5	0.053	42.5	0.7	0.4	4.0	0.048
65.....	46.0	1.0	0.2	4.1	0.061	41.7	0.8	0.7	5.5	0.050
75.....	44.1	0.8	0.4	4.0	0.058	39.3	0.2	0.0	4.2	0.062
85.....	46.0	0.7	0.3	2.9	0.062	40.0	0.1	0.0	2.1	0.060
90.....	47.0	0.5	0.8	5.4	0.044	42.6	0.5	0.4	6.0	0.040
95.....	47.3	0.9	0.8	5.0	0.045	42.6	0.5	0.4	6.0	0.040
60-45*	(47.0)	(0.9)	(0.6)	(4.5)	(0.053)	46.6	0.6	1.2	5.2	0.050
60-95*	(47.0)	(0.9)	(0.6)	(4.5)	(0.053)	45.0	0.7	0.4	4.8	0.050
90-60*	(47.0)	(0.5)	(0.8)	(5.4)	(0.044)	45.2	1.2	1.0	5.0	0.051
45-66*	(49.5)	(1.2)	(0.9)	(5.0)	(0.041)	46.8	0.7	0.4	4.1	0.050
(Initial trees Feb. 1).....	(52.8)	(1.4)	(1.0)	(4.8)	(0.040)					

\* Trees shifted March 17.

† Prior to March 17 the trees received no external nitrogen supply and contained no nitrate.

‡ No nitrate was found in the old roots of any of the trees at any time.

gaining, lost slightly in concentration of organic nitrogen (table VI). As in the case of the apple trees subjected to the same shift, the peach trees displayed a sudden and temporary increase in rate of growth of terminal leaves and stem tips, coupled with increase in soluble nitrogen in these organs shortly following the transfer from 65° to 95°.

In the consideration of nitrate assimilation in peach, it was stated that apple and peach (of the varieties employed) were apparently similar in their ability to synthesize organic nitrogen at the respective sand temperatures. The data of table II would seem to support this statement. Added nitrate resulted in some increase in concentration of organic nitrogen at each temperature, and taking current roots and old roots together (tables V and VI) this increase was greatest at 65°, lower at the two extremes of temperature, and otherwise followed closely in trend the curve for absolute reductase activity in apple (fig. 11). So also did the increase in amount of top growth (fig. 11, table II) which resulted from added nitrate as well as the absolute amount and percentage of organic nitrogen in the tops, analyses of which are not reported in detail.

At 65° and lower, added nitrate was definitely associated with increase in amount of current root growth (table II). In fact at 45° no new roots developed on the series lacking nitrogen in the culture medium. There were many root primordia present in both series, however, although in the latter group they failed, during the period of these experiments, to emerge through the periderm sufficiently to be readily noticeable macroscopically. A reason for this effect of added nitrate was not established, but assimilation of nitrate makes available materials which are essential for the development of new tissues such as amino acids and other relatively simple soluble forms of organic nitrogen. In the lack of new synthesis from inorganic nitrogenous nutrients, amino acids or related compounds are derived solely from reserves stored in initially present tissues. The assimilation of nitrate thus furnishes an additional source of organic nitrogen which conceivably might result in greater root growth, particularly at lower temperatures when there were present carbohydrates in abundance (table V).



## CARBOHYDRATES IN ROOT SYSTEMS OF APPLE AND PEACH

Whereas at lower temperatures the yield of current roots was increased by added nitrate, the same nutrient treatment at 75° and higher was associated with a decrease in amount of roots produced as compared with comparable cultures lacking nitrate. In fact at 90° many of the current roots died following application of the complete nutrient solution, but persisted in case of the cultures grown continually with no external source of nitrogen. Applications of nitrate in cases where it was assimilated have frequently resulted in lessened growth and even in death of the plants when available carbohydrates were limited in amount, as is frequently the case at relatively high temperatures (12, 18). In the reduction and assimilation of nitrate there necessarily occurs oxidation of carbohydrates or their derivatives, resulting in a decrease of those present unless supplied by new synthesis. In the current roots at 75° and higher, carbohydrates were definitely lower than in comparable trees that were grown with no external source of nitrogen (table V). With assimilation of nitrate at 90°, available carbohydrates became practically exhausted and death of the roots occurred.

Without knowledge of the anatomy of the root systems concerned, the results on concentration and quality of carbohydrates in the current roots would seem impossible of accurate interpretation. Of course, as already explained, added nitrate resulted in a decrease in percentage of carbohydrates, but the several analyses of apple and peach were remarkably consistent in showing that with a given nutrient treatment sugars were highest at low temperatures (tables III, V), and that there was directly correlated with increase in temperature a marked decrease in concentration of sugars. These results seem reasonable enough in view of the fact that the rate of respiration of the current roots was undoubtedly less at lower than at high temperatures. At least this was found to be true in the aerial organs of peach by actual measurement of the rate of carbon dioxide exchange (23), and the pH of the root tissues as already recorded was in harmony with this view. As will be shown later, however, differences in rate of respiration did not furnish a complete explanation for the accumulation of sugars at low temperature.

In contrast, starch was relatively low in the current roots of both genera at 45°, 50°, and 55°. This does not mean that condensation to starch cannot occur at these temperatures, but rather that the roots continually under those conditions had produced comparatively little potential starch storing tissue. The juvenile cortex which contributed in such large part to the samples as analyzed at low temperatures was apparently not a starch storing tissue. Only occasional small grains of starch were noticed in the cortex, none in the extensive embryonic zone, and few in the relatively immature stele. On the other hand, when some of the trees of both genera were shifted from 60° to 45°, starch accumulated in the current roots in much higher concentration than in any other groups of cultures. But the roots at 60° exhibited at the time of shift much more secondary phloem and xylem parenchyma, both potential starch storing tissues, than was observed in the roots continually at lower temperatures. At 45° starch as well as sugars has frequently been found to accumulate in the current roots of apple and peach when there were initially present potential starch storing tissues (19, 22, 23).

At temperatures of 60°, 65°, and 75° starch was found in moderate concentration (tables III, V) in the current roots of both genera, but as already mentioned there was present a considerable amount of potential starch storing tissue. Sugars on the other hand were low, as expressed on the basis of the percentage present in the entire current root system, and most of the reducing sugars and sucrose were found principally, not in the older secondary tissue, but in relatively meristematic elements in the vicinity of the cambium and embryonic zone of the root tips. The concentration of sugars in such cells did not appear to be much, if any, lower than at 50° or 55°, but the proportionate amount of meristematic tissue notably high in sugars was much greater at low than at higher temperatures (figs. 6-9). Hence the gross percentage data as it appears in tables III and V might well give an erroneous impression in the lack of anatomical evidence.

There was little meristematic tissue at 85° and 90° (fig. 9), which, coupled with an undoubtedly high rate of respiration (in cells containing respirable material), contributed to the very low percentage

of sugars as given for apple and peach in tables III and V respectively. The rather high percentage of starch in the current roots of peach at 85° is in contrast to apple where starch was relatively low. However, microchemical observations of the former genus were in accord with the results of macroanalysis. Starch grains persisted in the mature secondary parenchymatous elements of phloem and xylem long after sugars could be detected only in and near the embryonic zone of the root tip and in the very meager cambium region (fig. 9). It appears not improbable that enzymatic hydrolysis of starch was adversely affected at 85° in peach, although as will be shown presently this was seemingly not true in the old initially present roots. In any event, as the tissues of the current roots differentiated there was complete loss or disorganization of the more or less optically opaque protoplasm, and there was left the residue of starch as indicated in table V. This point will be discussed further.

As might be anticipated from the anatomical change already recorded, the shift from 60° to 95° resulted in a marked drop in sugars in the current roots of both genera. Accompanying this decrease and loss in amount of meristematic tissue there was an increase in rate of differentiation, with concomitant lignification and development of fibers, necessarily at the expense of sugars or their derivatives since cell walls in their various forms are condensation products of the simpler carbohydrates. Closely associated with these changes there was loss of starch, the hydrolysis of which appeared to continue in the respective cells as long as there could be detected any protoplasm. However, even after sugars were practically exhausted, presumably through respiration and contribution to cell walls (and in some degree through nitrate assimilation where nitrate was present), starch grains were still left undigested in cells apparently inert, although not always devoid of all other contents.

Following the shift just mentioned, there were left in the cortex and in the secondary parenchymatous tissues of the stele occasional cells containing materials other than and in addition to starch. These cells were present singly or in groups and were as a rule surrounded by optically empty tissue. The contents of such cells appeared more or less granular, had a pH value of 4.4-4.2, usually con-

tained many calcium oxalate crystals, and gave positive reactions for proteins, various nutrient elements, and tannins. Protoplasm of vigorous meristematic tissues did not give positive reactions to the usual protein reagents without preliminary denaturation, but the contents of the cells referred to reacted to most protein tests without preliminary treatment. It would seem, therefore, that a temperature of  $95^{\circ}$  resulted in disorganization of the protoplast of occasional cells whose contents (with the possible exception of sugars) remained unavailable to developing tissues of the current roots. This occurred in both apple and peach.

Cells of similar description were present at  $90^{\circ}$  and  $85^{\circ}$  but were noticed chiefly in the cortex as it began to turn brown. Such cells remained as described even after death of the cortex. At  $75^{\circ}$  cortical tissue occasionally exhibited cells of this type, whereas such cells were absent at the lower temperatures employed.

Little need be said concerning the percentage dry matter in the current roots of apple and peach (tables III, V). Proceeding from low to high temperature there was a consistent increase in such percentage. That this was not due in any considerable part to accumulation of sugars and starch would seem to have been made sufficiently clear. It was undoubtedly due principally to the definitely higher proportion of lignified xylem and mechanical elements which occurred with increase in temperature (figs. 6-9) and which necessarily developed through condensation of sugars or their derivative.

The concentration of carbohydrates in the old initially present roots of apple and peach may be mentioned briefly (tables IV, VI). The percentage of sugars showed considerable fluctuation, but in general it was found to be highest at lower temperatures, presumably because there was less vigorous respiration and because less carbohydrate material was required for the limited growth of roots that occurred at  $45^{\circ}$ ,  $50^{\circ}$ , and  $55^{\circ}$ .

The figures for starch and dry matter in the initially present roots are also similar in trend to those for sugars, with one significant exception. The lowest percentage of both starch and of dry matter in the old roots of apple and peach was at  $85^{\circ}$ , not at  $90^{\circ}$  or  $95^{\circ}$ . At the two higher temperatures, as verified by repeated microscopic observation, starch was present in high concentration in

parenchymatous tissues. Obviously under the conditions of these experiments the temperatures of  $90^{\circ}$  and  $95^{\circ}$  seriously limited digestion of starch in the old roots.

The tops of the apple and peach trees were not analyzed macrochemically for carbohydrates, but during the two months' period of these experiments there was found to be an abundance of sugars and starch in the old stems in all cases. This was likewise true for the current top growth with the following exceptions: at  $45^{\circ}$ ,  $50^{\circ}$ ,  $90^{\circ}$ ,  $95^{\circ}$ , and occasionally at  $85^{\circ}$ , slight wilting of leaves sometimes occurred during the middle of the day. This apparent lack of water was undoubtedly associated with the meager development of current roots at the temperatures mentioned. Although it has already been stated that these trees took in considerable water through their old roots, the photosynthetic activity of the trees was seemingly limited. At least the leaves and current stems were relatively low in sugars and starch.

### Summary

Young Stayman apple trees and similar Elberta peach trees were obtained from a commercial nursery in the fall of 1933. The tops were pruned back severely, all small roots were removed, the trees were planted in white quartz sand in self-draining culture jars and held in storage at  $35^{\circ}$  F. until January 31. During that period the sand was kept moist with tap water and by the weekly application of a nutrient solution complete except for nitrogen. On January 31 when experimental treatments commenced the buds had not expanded noticeably and no new roots were present.

For the following experimental period of two months the trees were grown in the original containers without transplanting, but were shifted to water baths so that some of the trees of each group were held continuously at sand temperatures of  $45^{\circ}$ ,  $50^{\circ}$ ,  $55^{\circ}$ ,  $60^{\circ}$ ,  $65^{\circ}$ ,  $75^{\circ}$ ,  $85^{\circ}$ ,  $90^{\circ}$ , and  $95^{\circ}$  F. respectively. The air temperature was in all cases about  $60^{\circ}$  to  $65^{\circ}$  at night and  $65^{\circ}$  to  $70^{\circ}$  during the day. Temperatures mentioned in the following paragraphs refer to the temperature of the sand in which the roots were grown.

1. In both apple and peach the maximum yield of current roots and of current tops occurred at a sand temperature of  $65^{\circ}$ , and with increase or decrease in the temperatures employed there was a de-

crease in yield of roots and aerial organs. At  $45^{\circ}$  root primordia were present but few roots emerged through the periderm during the period of these experiments. At  $95^{\circ}$  no new roots appeared and the old roots eventually died.

2. At  $65^{\circ}$  and lower temperatures the newly developed roots of both genera were typically white, of relatively large diameter, extremely succulent, lacking in mechanical strength, and they characteristically exhibited few fine laterals.

3. In contrast, at  $75^{\circ}$  in both genera the cortex turned brown and gradually sloughed off. The remainder of the root, the central cylinder, was typically very woody, of considerable mechanical strength, and lacking in succulence. There were present many fine lateral roots. At  $85^{\circ}$  and  $90^{\circ}$  the roots were similar in quality but much smaller in diameter and less extensive.

4. The initially present pruning wounds of the old roots of both apple and peach callused over earliest at  $85^{\circ}$  and  $90^{\circ}$ . At the latter temperature there was often incomplete covering of the pruning wounds by callus tissue and the callus became completely suberized when only a few cells thick, cell division having ceased. There was in general at  $85^{\circ}$  complete covering of the pruning wounds by a rather thin layer of callus which suberized and turned brown rapidly. At  $75^{\circ}$  wound callus tissue was thicker and remained white for a somewhat longer time. At  $65^{\circ}$  and lower the callus tissue developed more slowly, failed to mature, was white and fluffy in appearance, and broke off easily in handling. More or less satisfactory callusing occurred at  $85^{\circ}$ , therefore, a temperature at least  $10^{\circ}$  higher than that which could be considered favorable for root growth under the conditions of these experiments.

5. In both genera at  $65^{\circ}$  and lower there was present directly back of the root tip an extensive zone of embryonic tissue, the cortex persisted for the duration of these experiments, the pericycle although exhibiting considerable cell division did not become suberized externally, nor were there produced any pericycle or phloem fibers. At several centimeters from the root tip there was also present in transverse section a wide band of undifferentiated tissue in the region of the stelar cambium, but development of secondary tissues and lignification of xylem were notably slow. These factors were therefore as-

sociated with the general condition of succulency and lack of mechanical strength.

6. At 75°, in case of both apple and peach, the embryonic zone of the root tip was much less extensive than at lower temperatures, owing to relatively early differentiation of tissues which was soon followed by the development of secondary elements and death of the cortex. Accompanying maturation of the juvenile cortex there was developed an active cork cambium that produced externally cells that suberized rapidly, and internally parenchymatous cells and many heavy walled fibers. The stelar cambium was likewise active and secondary phloem and xylem were extensive. Conspicuous elements of the phloem were the large bundles of strongly developed fibers, and, with the exception of ray cells, the entire xylem was thick walled and lignified. These factors were obviously associated with the general lack of succulence and considerable mechanical strength of the roots concerned.

7. At 85° and 90° the primary tissues were made up of a comparatively small number of cells and differentiation occurred even earlier in the roots of apple and peach than at 75°. The embryonic zone of the root tip was therefore extremely limited in volume. The cortex died early and the pericycle produced externally a few cells that suberized rapidly, although frequently the periderm was non-continuous especially at the higher temperature. There were present a few small fibers in pericycle and phloem, but the amount of phloem and xylem was limited, first by the relatively small number of cells in the meristem of the root tip and second by a stelar cambium region only two or three cells wide in transverse section. In fact mature phloem cells and strongly lignified xylem elements frequently abutted one upon the other. These factors therefore contributed to the small diameter of the roots concerned and to their extreme lack of succulence.

8. Respective differentiated tissues of the current roots of the two genera were not noticeably different in H ion concentration under comparable conditions. Young cortical cells were approximately pH 5.2-4.8, but with rapid maturity of the cortex at temperatures of 75° and higher the cells became more acid (4.4-4.2). Cellulose walls apparently failed to react with the usual indicators. Lignified walls

gave a so-called pH value of 4.4-4.0. At 75° and higher a relatively large percentage of the current root system consisted of lignified xylem elements and fibers that invariably exhibited the acid reaction mentioned. The cell contents of phloem were much less acid. Recently developed cells had a pH value of 6.2-5.9, mature cells 5.2-4.8, and the phloem matured most rapidly at higher temperatures.

Proceeding from low to high temperatures the embryonic tissues of cambium and root tip became increasingly acid in reaction. This was apparently associated with relatively rapid respiration and accumulation of carbon dioxide in the tissues concerned. The real pH of meristematic tissue was therefore problematical, but the pH was under all conditions much less acid than that of mature xylem walls and seemed to approach the more nearly alkaline condition of young phloem cells (6.2-5.9) at temperatures of 65° and lower. On the other hand, at 90° the pH of meristematic tissue was probably about 5.0-4.8. The rapidly maturing non-succulent roots at temperatures of 75° and higher were therefore low in proportion of less acid tissues.

9. Nitrate was freely *absorbed* by the current roots of apple and peach at the respective temperatures employed, and without exception was limited exclusively to these organs for the duration of the experiments. Whereas the temperature of the nutrient medium affected little, if at all, the ability of the roots to absorb nitrate (provided they were not already saturated to capacity with it), temperature had a very marked influence on the ability of the roots to reduce nitrate to nitrite, ammonium, and amino acids. This process of reduction and synthesis with accompanying oxidation of carbohydrates or their derivatives may be included in the term *nitrate assimilation*.

10. ECKERSON'S (6) method was employed for determining the *reducase* value or comparative nitrate reducing ability of aqueous extracts of apple root tissue. Chemical analyses indicated that *reducase* activity furnished an index of the relative rate of assimilation of nitrate. Certain samples that consisted principally of the relatively meristematic tissue of the root tips were tested for *reducase*. Proceeding from low to high temperature the results showed that there was a consistent increase in rate of nitrate reduction. But at



higher temperatures there was present relatively little meristematic tissue and it was comparatively more acid than at lower temperatures, a fact in itself unfavorable for reducase activity. At 65° and lower the intensity of nitrate reduction in the root tips was much less than at higher temperatures, but samples even from the proximal or oldest end of the current roots at 65° contained a fairly high proportion of active cells and exhibited about the same degree of reducase activity as the tips.

Coupled with this high proportion of active, less acid tissues and a relatively low percentage of inert, more acid elements such as mechanical fibers, lignified xylem, and strongly suberized periderm, there was produced at 65° the greatest yield of roots and aerial organs. Consequently it seemed logical to find in the current roots of the apple trees at 65° the highest absolute reduction of nitrate as computed on the basis of reducase activity. It should be recalled, however, that these roots were lacking in mechanical strength.

11. Macro- and microchemical analyses of apple trees corroborated the statements in the foregoing paragraph, and similar analyses of peach trees before and after addition of nitrate indicated that assimilation of nitrate was essentially similar in both genera at the respective temperatures employed. With reduction of nitrate there necessarily occurs oxidation of carbohydrates or their derivatives. Analyses ultimately showed that where nitrate was reduced carbohydrates were lower than in comparable cultures which received no external source of nitrogen.

12. At 65° and lower the current roots of both apple and peach were notably high in percentage of organic nitrogen. This was intimately associated with the relatively large proportion of meristematic tissue present at these temperatures. At higher temperatures there was proportionately less such tissue and the concentration of nitrate-free nitrogen was extremely low.

13. The trees of both genera which were grown continually at 55° or lower were high in percentage of reducing sugars and sucrose but low in starch. However, the roots of these trees were high in proportion of comparatively meristematic tissue that contained sugars but did not store starch. Likewise the persistent juvenile cortex which was not a potential starch storing tissue contributed in

large part to the aliquots analyzed macrochemically. On the other hand, when some of the trees of both genera were shifted from 60° to 45° starch accumulated in the current roots of both groups in much higher concentration than in any other case, but the roots at 60° exhibited at the time of shift much more secondary phloem and xylem parenchyma, both potential starch storing tissues.

14. At 75° and higher the percentage dry matter in the current roots of both genera was high. This was intimately associated with the presence of thick walled lignified xylem and mechanical fibers. Sugars and starch were low, probably in part because of a comparatively high rate of respiration, but the development of thick walled fibers and strongly lignified xylem walls was necessarily at the expense of sugars or their derivatives, as cell walls in their various forms are condensation products of carbohydrates.

15. The current roots of both genera were extremely deficient in sugars and starch at 85° and 90°. The old initially present roots of the trees at 90° and 95° contained, however, a very high concentration of starch. This temperature apparently seriously limited digestion of starch in the old roots.

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# MORPHOLOGY AND BIOLOGY OF SOME SPECIES OF ODONTIA

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(WITH NINETEEN FIGURES)

## Introduction

The Hydnaceae, as recognized by most students of the group, is a family of the Hymenomycetes containing a heterogenous group of pileate, sessile, reflexed, and resupinate plants, characterized by the possession of teeth or spines, granules, and warty protuberances which usually point downward. These last are covered by a hymenium which usually does not extend over their apices.

Considerable intergradation occurs within the family. As a result much confusion in classification has arisen because of the difference in emphasis placed on the various diagnostic characters by different students or even by the same investigator in the course of his studies.

In 1929 the late Professor C. H. KAUFFMAN suggested the desirability of a cultural study of this group. The classification of the species collected for this study necessitated a review of the whole taxonomic situation and a comparative study of the morphology of the species. A start has been made in the study of the biology of the resupinate species of the Hydnaceae. Eleven species have been grown and their development compared on synthetic media. It is hoped that the information thus obtained will help to throw light on the relationships in the group and lead to a more usable and stable classification.

Although much attention has been devoted to the taxonomy of the Hydnaceae, comparatively slight attention has been given to a study of them in pure culture. Difficulty has been experienced in germinating the basidiospores of species in the Hydnaceae. When germination was secured, the cultures usually remained sterile or produced structures which were described as bulbils, oidia, or chlamydospores. In only a few instances were basidiospores obtained.

BREFELD (6) was the first to study species of this family in culture.

He reported difficulty in securing germination of basidiospores in the genera *Sistotrema*, *Hericium*, *Hydnum*, *Mucronella*, *Odontia*, and in the species *Grandinia granulosa* Fr. He succeeded in germinating the spores of *Kneiffia setigera* Fr., *Grandinia crustosa* (Pers.) Fr., and *G. mucida* Fr. His cultures of these all remained sterile. The basidiospores of *Phlebia merismoides* Fr., *Ph. radiata* Fr., *Ph. contorta* Fr., and *Ph. vaga* Fr. were easy to germinate and oidia were produced in eight days. The spores of *Radulum orbiculare* Fr., *R. pendulum* Fr., *R. fagineum* (Pers.) Fr., *R. molare* Fr., and *R. lateum* Fr. germinated easily. The side branches of the mycelium became constricted into beadlike chains which he did not consider typical conidia. Only *R. pendulum*, now believed to be a tuberculate form of *Corticium subcostatum* Karst., produced basidiospores in his cultures.

FALCK (17) reported fruiting of *Phlebia merismoides* in cultures, not only from basidiospores but also from a single oidium.

LYMAN (30) studied a great number of Hymenomycetes, especially species in the Thelephoraceae. He apparently also studied many of the Hydnaceae but unfortunately was unable to give much information concerning this group because he lost his data. He reported, however, that chlamydospores were obtained for this family. HORTON (23) studied the production of bulbils and similar propagative bodies in the Hymenomycetes. He found bulbils, oidia, and basidia in cultures of *Grandinia crustosa* (Pers.) Fr., now *Odontia crustosa* (Pers.) Quél.

DESEYNES (15) and PATOUILLARD (36) observed macro- and micro-conidia in collections of *Hydnum erinaceus* Bull. and *H. coralloides* (Scop.) Fr. They mentioned the resemblance of micro-conidia to basidiospores, which, as will be shown later, they probably were.

BROOKS (7) cultured *Hydnum coralloides* on ash and secured the formation of small but typical fructifications in about four months' time. Further cultures produced abnormal fruit bodies with rudimentary spines. He secured this fruiting from spores which were collected on a sheet of clean glass and transferred to tubes containing the wood.

#### Methods of study

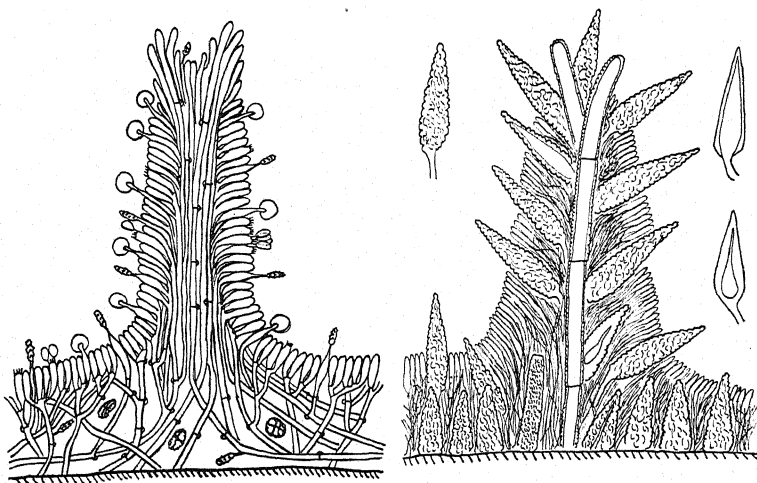
BURT (8-11) suggested a standard method of procedure for the study of specimens of the Thelephoraceae. Briefly this consists in

moistening a bit of the hymenium with alcohol, wetting it with water in order to section the material easily, then transferring the sections to 7 per cent potassium hydroxide solution to expand the tissue to nearly natural size. In some instances lactic acid was used because potassium hydroxide darkened the sections. Permanent slides were made by staining the sections with water soluble eosin, then mounting them in dilute glycerin and, when ready, cementing the coverglasses to the slides. This is essentially the procedure followed by OVERHOLTS (35). He acidified the eosin stained sections with acetic acid so that the color would not fade so readily. BOURDOT and GALZIN (5) and DONK (16) used potassium carbonate solutions colored with eosin, and chloral hydrate with iodine in their investigations of the Hymenomycetes.

In recent years several European investigators have studied the effects of various reagents on fungi, with the result that the reactions have been utilized by some as important diagnostic characters. BOURDOT and GALZIN (5) found that the use of potassium carbonate with eosin stained the protoplasts of certain types of mycelia more readily than others and used this as a supplementary character in delimiting species. Gloecystidia are also more easily detected by the use of potassium carbonate with eosin or chloral hydrate with iodine. KÜHNER (27) has even gone so far as to use such reactions as a means of distinguishing genera. He maintains that the morphological characters alone are insufficient to separate the genera *Lentinellus* Fayod and *Lentinus* Fr., and has emended the former by recognizing the amyloid spore as the principal diagnostic character. In the Hydnaceae, the genus *Dryodon* (*Hericium*) is also characterized in part by the amyloid spore character. A striking reaction is noted by BOURDOT and GALZIN (5) in *Odontia* (*Acia*) *uda* (Fr.) Bres., which turns purple when exposed to the fumes of ammonia. They consider this a reliable means for separating this species from *O. (Acia) fusco-atra* (Fr.) Bres.

Different workers have used reagents of various kinds and strengths. The present studies show the necessity for giving the strengths of reagents used. For instance, a careful examination of sections from fresh specimens of *Odontia arguta* (L.) Quél. shows small capitate structures, herein called "capitate cystidia," which at

first may easily be mistaken for air bubbles or drops of oil in sections which have not been fully expanded or which contain air (fig. 1). Once this structure is recognized, it is easily distinguished. However, Amann's mounting medium, chloral hydrate with iodine, 95 per cent alcohol, and 0.5 per cent potassium hydroxide all rapidly dissolve the globose cap from the capitate cystidium, leaving a small, cylindrical, hyphal stalk. Although 5 per cent potassium carbonate also dissolves the cap, it acts more slowly.



FIGS. 1, 2.—Fig. 1 (left), diagrammatic sketch showing structures of spine, axillary hyphae, and capitate and oxalate incrustated cystidia. Fig. 2 (right), same showing structure of spine, incrustated axillary hyphae, imbedded and projecting cystidia. Sketch at right of spine shows cystidia after incrustations were dissolved.

Inasmuch as several of the common reagents will alter certain of the diagnostic characters, it is imperative that at least a preliminary examination of the material be made in distilled water and that the reagents be then added by drawing them under the coverglass with a piece of filter paper while the mount is under observation.

Studies of dried specimens, following the procedure outlined by BURT (8-11) or OVERHOLTS (35), may sometimes fail to reveal the presence of diagnostic characters, as 7 per cent potassium hydroxide and 95 per cent alcohol have been found too strong, destroying or



obscuring certain types of structures found in Basidiomycetes. This may account for some of the discrepancies for the same species between BURT's descriptions and those of some European workers, especially with reference to the presence or absence of incrustations on the hyphae and cystidia.

The following technique has been employed in studying specimens. Sections were mounted in distilled water for an examination for the presence of cystidia, cystidium-like organs, and incrustations. They were then treated either with 0.5 per cent potassium hydroxide or with 5 per cent potassium carbonate colored with eosin to expand and stain the spores and tissues. In the case of material which did not section readily, a small piece was soaked in water for half an hour or longer, until it sectioned easily.

Chloral hydrate with iodine, as used by STEVENS (39), was found to be a satisfactory reagent to follow distilled water when a detailed study of the section was wanted, inasmuch as it expands the tissues better than either potassium hydroxide or potassium carbonate. However, it has some solvent action. The iodine in this solution gives a yellow-brown color to the hyphae and a darker brown color to the gloecystidia. Amyloid spores of course turn blue. Sometimes the definition of an object expanded with this reagent is unsatisfactory, and in those cases the 5 per cent potassium carbonate with 0.01 per cent eosin was used. This dye stains the hyphae so that a sharp definition can be secured. The use of Amann's mounting medium or 5 per cent potassium carbonate with various dyes such as cotton blue or eosin has been helpful in distinguishing individual hyphae. As yet no one reagent has been found which is entirely satisfactory for the study of all specimens.

A solution of 10 per cent hydrochloric acid was used to remove the calcium oxalate, and with this obstructing material dissolved the structure of the specimens can be more readily ascertained.

It is difficult to make tissue cultures of the resupinate Hydnaceae, except in the case of a few species which have rhizomorphs, because the subiculum and teeth are thin. Only one tissue culture was secured. *Odontia fragilissima* (B. & C.) Brown was cultured from a rhizomorph after the usual procedure of surface sterilization in mercuric chloride followed by several changes in distilled water.

Specimens were collected in the field and wrapped in waxed paper. A spore fall was obtained by placing a specimen over sterile petri dishes or capsules. When the deposit of spores was visible (after 2-12 hours) sterile distilled water was added. The proper dilution was determined by making a fine spray on a glass slide and examining individual drops to see that the number of spores did not exceed two or three per drop. The spore suspension was sprayed on previously poured agar plates as described by KAUFFMAN (24). After germination, which takes 12-72 hours at a room temperature of approximately 20° C., germinated single spores were isolated by cutting out small blocks of agar 1 mm. square under the low power of the microscope. The spore was transferred to a sterile agar plate or to a slanted test-tube. Difficulty similar to that reported by BREFELD (6) was experienced in securing the germination of spores. Spores from field material germinated in only 17 instances out of 155 attempts. Germination from basidiospores produced in culture was much more certain.

Experiments were conducted using a maltose agar,<sup>1</sup> LEONTIAN'S agar (28), KOTILA'S agar (26), a 6 per cent oatmeal agar, and a 2 per cent malt extract agar. KOTILA'S agar was found to be the most satisfactory for vegetative growth and fruiting, inasmuch as basidiospores formed on this substratum 5-10 days sooner than on the others. Therefore this was used as the standard medium on which these fungi were grown. KOTILA'S agar did not always solidify after the usual sterilization at 15 pounds' pressure for 15 minutes in the autoclave, and this difficulty was overcome by increasing the quantity of the agar from 20 to 30 gm. per liter of water or by sterilizing the agar at 10 pounds for 20 minutes.

The various species were also grown on filter paper pads saturated with the different nutrient solutions used for these agars. Filter paper was cut and folded into U-shaped pads and placed in an inverted position in small glass capsules, then dry-air sterilized.

Some of the species studied readily formed a hymenium in about a month after isolation of the single spores. Other species would not

<sup>1</sup> KAUFFMAN'S maltose agar—to the liter: maltose 5.0 gm., peptone 0.1 gm., magnesium sulphate 0.5 gm., potassium di-hydrogen phosphate 0.25 gm., calcium nitrate 0.1 gm., and agar 15 gm.

fruit readily in culture, and in these instances, fruiting could sometimes be brought about by the following technique. The fungi were grown in glass capsules on filter paper pads, moistened with different nutrient solutions. After sufficient mycelium was formed (10 days or more), the nutrient solution was replaced with sterile distilled water. Fruiting occurred 10–20 days later, depending upon the species.

Sterilized thin slabs of white pine, basswood, and elm were also used in glass capsules with a nutrient solution, or with their respective wood decoctions.

Approximately 600 collections of resupinate Basidiomycetes were made. Many of these were sent to European workers for identification. ABBÉ H. BOURDOT and M. A. DONK both have identified specimens, and have given many helpful comments, this assistance being of great value. In the following discussion, the citation of the author's collection number indicates that a duplicate of that specimen has been deposited in the Herbarium of the University of Michigan.

The following species were studied and cultures were secured from those marked with an asterisk. All the specimens cultured were collected in the vicinity of Ann Arbor, Michigan, with the exception of *Odontia bicolor* from Louisiana.

- \* *O. arguta* (Fr.) Quél.
- \* *O. fusco-atra* (Fr.) Bres. (three collections)
- \* *O. uda* (Fr.) Bres.
- \* *O. stenodon* (Pers.) Bres.
- \* *O. brinkmanni* Bres.
- \* *O. hydroides* (C. & M.) v. Höhn.
- \* *O. bicolor* (A. & S.) Bres. (from Louisiana)
- \* *O. fragilissima* (B. & C.) Brown
- \* *O. separans* (Peck) Brown
- O. himantia* (Schw.) Bres.
- O. fimbriata* Fr.
- O. setigera* (Fr.) Miller

### Results

*Odontia arguta* (figs. 1, 3, 4, 5, 6, 7)

*Odontia arguta* (Fr.) Quél.,—Fl. Myc. Fr. 435. 1888.

BRESADOLA, Atti. Accad. Rovereto III. 3:98. 1897.

BOURDOT & GALZIN, Hym. de France p. 427.

Fructification effused in patches up to 60 cm. long by 30 cm. wide, usually smaller, soft, arachnoid to tomentose, thin, adnate, cracking on drying, white to colonial buff (MP)<sup>2</sup> when fresh, darkening slightly when dry; margin indeterminate, pubescent; spines concolorous with the hymenium, tips lighter, granulate to cylindrical subulate, sometimes slightly flattened and connected at base, crowded, 1-3 mm. long, apices penicillate; hyphae thin walled, 2-4  $\mu$  in diameter, loose in subiculum, more compact under the hymenium and in axes of the spines, clamp connections present; basidia 10-16 $\times$ 3-5  $\mu$ ; spores hyaline, smooth, variable from broadly oval to subglobose, often apiculate, 3-5 (6) $\times$ 3-4.5  $\mu$ ; capitate cystidia arising from the basidial layer or from tramal hyphae, present on sides and between the spines, not in subiculum, the cap part 7-9  $\mu$  in diameter on a hypha 2.5-3  $\mu$  in diameter, cap soluble in alcohol, potassium hydroxide, chloral hydrate with iodine, hot water, and slowly so in potassium carbonate; the second type of cystidia (fig. 1) of two forms present in the subiculum and on the sides and apices of the spines: the first 1-2  $\mu$  in diameter, the second 3-5  $\mu$  in diameter, constricted near apex to 2  $\mu$  in diameter, the apices of both incrustated with calcium oxalate 2.5-3.5  $\mu$  in diameter; masses of calcium oxalate or other mineral matter soluble in hydrochloric acid often found in the subiculum.

This is by far the most common species of *Odontia* in the vicinity of Ann Arbor. It is found on the under sides of rotten logs of both deciduous and coniferous trees. In addition to specimens from Michigan, material from Canada, Iowa, New York, Pennsylvania, and

<sup>2</sup> The technical color names indicated by (MP) are from MAERZ & PAUL (31). Names followed by (R) are from RIDGWAY (38). The writer has used the recent publication of MAERZ & PAUL because RIDGWAY'S Color Standards and Nomenclature is out of print. Many of the names used by RIDGWAY are retained by MAERZ & PAUL, who also included in the index a definite reference to most of the RIDGWAY color names.

North Carolina has been studied, which indicates that it has a wide distribution in North America.

One of 15 attempts to culture this species was successful. The basidiospores germinated on agar plates in 3-5 days at room temperature. They produced a single germ tube which became branched within 48 hours. The mycelium was mostly submerged in the agar. On the surface it developed a thin compact film. The color varied from white to cream, and when fruiting, it became cream colored. Spores were produced after a period of 20-30 days. They germinated somewhat readily at the end of 3 days.

This fungus produced spines on nutrient agar (fig. 3), on filter paper pads (fig. 4), and on wood (figs. 5, 7). Nearly typical spines were produced on the under side of the filter paper pads (fig. 4), and branched upright structures were formed on the upper surface of filter paper pads, on agar, and on wood. The capitate cystidia and the oxalate incrustated cystidia, so characteristic of this species in nature, were not produced in culture.

Cultures grown in light or in darkness at room temperature did not show any appreciable variation in the rate of growth or fruiting.

This species is homothallic, as shown by the fact that 22 monosporous cultures produced teeth with hymenium, typical basidia, and basidiospores. Clamp connections were present on all mycelia.

*Odontia fusco-atra* (figs. 8, 9, 10)

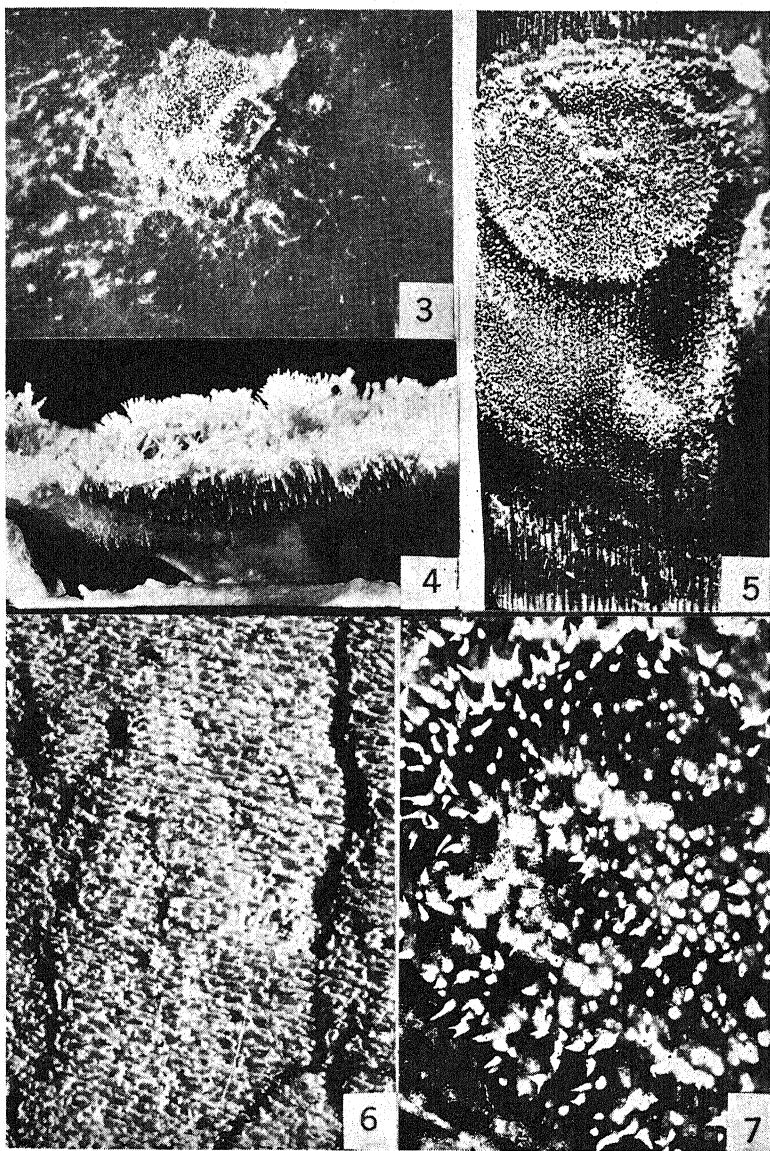
*Odontia fusco-atra* (Fr.) Bres.,-Atti. Accad. Rovereto III. 3:95. 1897.

*Acia fusco-atra* (Fr.) Pat.,-BOURDOT & GALZIN, Hym. de France p. 417.

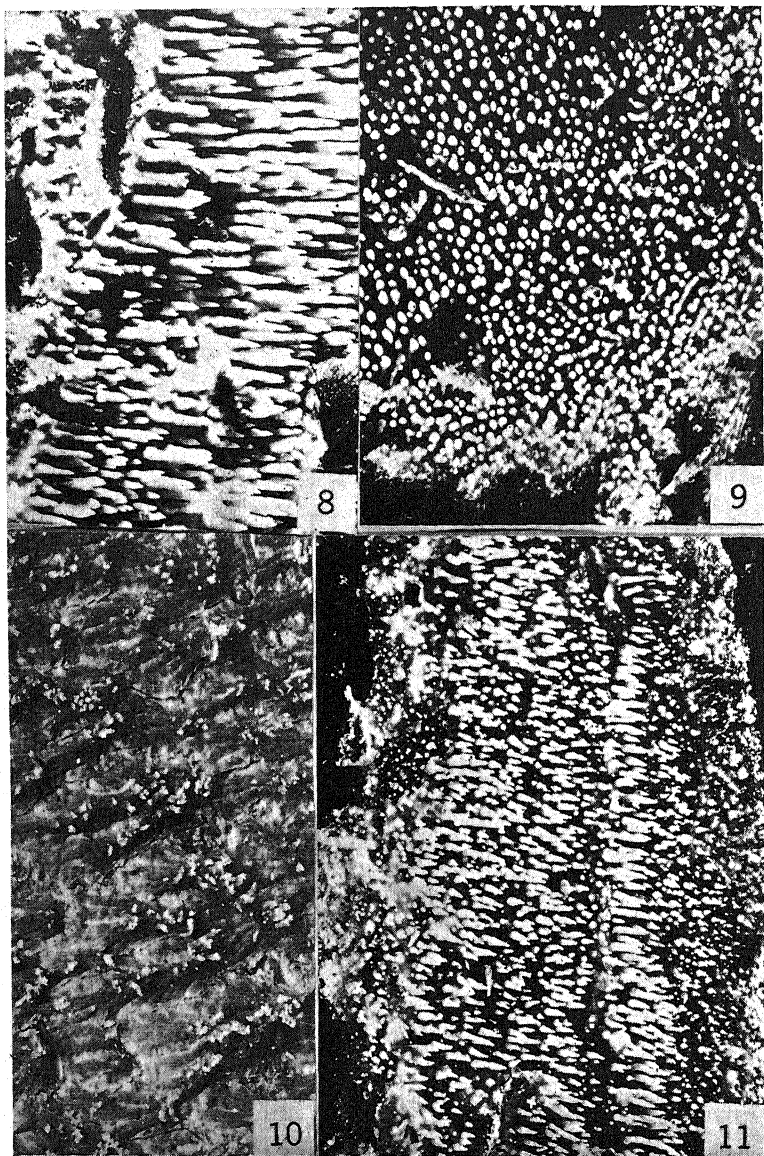
*Mycoacia fusco-atra* (Fr.) Donk,-Med. Ned. Myc. Ver. 18-20:152. 1931.

*Hydnum carbonarium* Peck,-N.Y. State Mus. Rept. 40. p. 55. 1887.

Fructification effused in patches up to 60×10-20 cm., thin, adnate, color variable from bluish white to peach beige (MP) to cinnamon brown (MP) to burnt umber (MP) when fresh, art brown (MP) to black when dry; margin bluish white to yellowish, pruinose or finely byssoid; spines slender, subulate, pointed or with apices di-



FIGS. 3-7.—*Odontia arguta*: Fig. 3, culture in petri dish on KOTILA's agar; natural size. Fig. 4, same on filter paper pad showing typical teeth produced on lower surface;  $\times 2$ . Fig. 5, same on pine wood; natural size. Fig. 6, fruit body (no. 426) on rotten hardwood. Ann Arbor, Mich.;  $\times 5$ . Fig. 7, culture showing detail of spines;  $\times 5$ .



FIGS. 8-11.—Fig. 8, fruit body of *O. fusco-atra* (no. 417) side view, on basswood. Collected near Ann Arbor, Mich. Fig. 9, looking down on tips of spines of same fructification. Fig. 10, dried specimen of same showing clumping of spines or “molariform” teeth. Photograph by EDGERTON. Fig. 11, *O. uda* (no. 426) collected near Whitmore Lake, Michigan.  $\times 5$ .

vided, concolorous with the subiculum or with tips lighter colored; subiculum hyphae moderately dense, becoming more compact just under the hymenium,  $2.5-3\ \mu$  in diameter, thin walled, with clamp connections; hyphae in axes of spines compact, parallel, ascending to somewhat interwoven; cystidia incrustated,  $5-8\ \mu$  in diameter, abundant in axes of the spines, usually imbedded, sometimes projecting from the spines at various angles; cystidioles occasionally present on the sides of spines, subulate, difficult to detect; basidia  $14-20 \times 3-4\ \mu$ ; spores oblong cylindrical, sometimes slightly curved,  $4-5 \times 2\ \mu$ .

Twelve collections from the vicinity of Ann Arbor (nos. 417, 425, 81) were studied and also one collection each from New York, Ohio, North Carolina, and Holland respectively. The New York collection (no. 34) was determined by BOURDOT.

The variations noted in the color of this species, when compared with the relative constancy of color in a number of other species of the group, suggested that two or more species might be involved. However, a study of two fresh collections showed a change in color from bluish white to cinnamon brown (MP) in less than an hour. The burnt umber (MP) color appeared in about two hours, and the dried collections varied as noted. Material has been collected in the field in which the specimens were uniformly cinnamon brown (MP) and uniformly burnt umber (MP). Other collections have shown a mottled appearance caused by transitional changes in different areas.

Fourteen monosporous cultures were made successfully from three specimens collected in 1929, 1930, and 1932. Monosporous cultures of these had clamp connections, produced basidia and basidiospores. Repeated isolations behaved likewise, thus establishing the homothallic nature of the fungus. A variation in the time it took monosporous cultures to fruit is worthy of mention. Fruiting usually occurred in 30 days at room temperature on maltose agar and in 20 days on KOTILA's agar. Some cultures, however, required three to four months to mature.

This species produced mostly submerged mycelium with merely a scant floccose film over the surface of the agar. The fructification was a yellowish crust on the surface of the agar and was composed of a compact palisade-like layer of basidia with here and there an up-



right incrustated hypha. These incrustated hyphae or cystidia were similar to those found in the axes of the spines, but differed in having larger granules forming the incrustation, and they usually occurred solitary. The striking color change exhibited by field collections was absent from cultures on agar or on filter paper pads.

An examination of the type of *Hydnum carbonarium* in the Herbarium of the New York State Museum resulted in finding that the specimen is a depauperate or young stage of *Odontia fusco-atra*.

*Odontia uda* (fig. 11)

*Odontia uda* (Fr.) Bres., -Atti Accad. Rovereto III. 3:97. 1897.

*Acia uda* (Fr.) B. & G., -Hym. de France p. 414.

*Mycoacia uda* (Fr.) Donk., -Med. Ned. Myc. Ver. 18-20:151. 1931.

Fructification effused, in small patches, 3-6 × 1.5-2 cm., thin, soft, waxy, adherent, sulphine yellow (R) to naphthalene yellow (R) when fresh; margin indeterminate, pruinose, whitish to concolorous; spines concolorous with the hymenium, slender, elongated, 0.5-1 mm. long, solitary or slightly confluent, crowded, apices entire or denticulate; hyphae of the subiculum very compact, 2.5-3  $\mu$  in diameter, flexuous, septa few; hyphae of the subhymenium thinner walled, abundantly septate, clamp connections present; hyphae in axes of the spines adherent, very compact, 3-4  $\mu$  in diameter; basidia 13-16 × 4-5  $\mu$ , clavate; spores 4-6 × 2.5-3  $\mu$ , hyaline, oblong cylindrical and often containing two shining drops; cystidioles subulate, projecting 6-10  $\mu$  beyond the hymenium, 2-3  $\mu$  in diameter. After rejuvenation the spines may be densely bristly with hyphae or cystidioles (?) 2-3  $\mu$  in diameter.

Nine collections were made in Michigan of which no. 36 was determined by BOURDOT. It occurs on rotten hardwood logs, and is found more frequently on *Fraxinus nigra* than on other hardwoods.

When in good growing condition this fungus has a strong anise odor and exhibits a striking color reaction to alkali (5). Treating sections with potassium hydroxide produces a reddish violet color. Exposure of pieces of a specimen to the fumes of ammonia produces a violet color. BOURDOT and GALZIN (5) have distinguished *Odontia* (*Acia*) *uda* from *O. (Acia) fusco-atra* by this reaction.

Six monosporous cultures were made of *Odontia uda*. The mycelium produced by the single germinated spores was  $2.5-4\ \mu$  in diameter, septate, and had clamp connections on the septa. Chlamydospores  $8-15\ \mu$  in diameter were abundant in the cultures. The basidia were oblong-clavate,  $15-30 \times 3-4\ \mu$  in diameter. All six monosporous cultures formed clamp connections, produced basidia and basidiospores, and *Odontia uda* is therefore a homothallic species.

Small penicillate spines were produced on the upper surface of filter paper pads moistened with different nutrient solutions. The spines consisted of clusters of spreading, upright, slightly incrustated hyphae. The lower part of these spines was covered with the hymenium. The cultures produced a very strong, characteristic odor of benzaldehyde rather than the anise odor of fresh specimens. There was no color reaction when the mycelia of the cultures were exposed to the fumes of ammonia as was noted for material collected in the field.

*Odontia stenodon*

*Odontia stenodon* (Pers.) Bres.,—Atti. Accad. Rovereto III. 3:96. 1897.

*Mycoacia stenodon* (Pers.) Donk,—Med. Ned. Myc. Ver. 18-20: 151. 1931.

*Acia stenodon* (Pers.) B. & G.,—Hym. de France p. 415.

*Oxydontia stenodon* (Pers.) Miller,—Mycologia 25:294. 1933.

Fructification effused, in patches  $10-15 \times 8-10\text{ cm.}$ , adnate, waxy, cracking when dry, white to yellowish, pale salmon (R) when fresh, drying with yellow and reddish colors; margin fibrillose, radiating, narrow, white; spines often nodose, clustered or crowded, slender, connate at base, 1-3 mm. long; hyphae of subiculum very compact, agglutinated,  $2.5-4\ \mu$  in diameter, moderately thick walled, the structure difficult to distinguish; hyphae in axes of spines compact, agglutinated,  $3-4\ \mu$  in diameter, prolonged into sterile point or sometimes divided, apparently not incrustated, clamp connections present; basidia clavate,  $15-24 \times 3-5\ \mu$ , 2-4 sterigmata  $2.5-3\ \mu$  long; no cystidia; cystidioles  $2.5-3\ \mu$  in diameter on a level with the basidial layer or projecting  $2-5\ \mu$  beyond; spores hyaline, oblong allantoid,  $3.5-5.5 \times 2.5\ \mu$ .

Three collections of this fungus were studied; one from New York, no. 35, which was determined by BOURDOT, and two from Michigan.

Both two- and four-spored basidia were found side by side in the hymenium on the same tooth.

A multisporous culture produced very little aerial mycelium but typical teeth were formed on the agar. Structurally the teeth were identical with those produced in nature. The hyphae of the submerged mycelium ranged from 1.5 to 4  $\mu$  in diameter, septate, with clamp connections. Incrusted, aerial hyphae were produced.

*Odontia brinkmanni*

*Odontia brinkmanni* Bres.,—Ann. Myc. 1:88. 1903.

*Grandinia brinkmanni* (Bres.) B. & G.,—Hym. de France no. 321.

Bull. Soc. Myc. de France. 1914.

Fructification effused, forming patches 10–20 cm., thin, pruinose, adherent, white when fresh becoming yellowish on drying; margin subindeterminate; spines hemispherical, crowded, often conical and elongated; hyphae of the subiculum moderately loose, 1.5–4.5  $\mu$  in diameter, thin walled, often collapsing, clamp connections present; basidia urn-shaped, 4–8 spored with short sterigmata; spores hyaline, smooth, subelliptical, 4–6  $\times$  2–2.5  $\mu$ ; no cystidia; oxalate crystals abundant in the subiculum and spines.

This species was collected twice in Michigan, and one collection (no. 27) was determined by BOURDOT. It occurs on rotten hardwood logs.

In culture it produces a white to cream colored mycelium, at first chiefly submerged, later aerial and floccose. The mycelium of multisporous origin had clamp connections; however, it never produced basidia and basidiospores. Beadlike chains of thick walled chlamydospores were developed. Single chlamydospores were isolated and the mycelium produced was septate with clamp connections, white, and more floccose from the start than that produced from the basidiospores. However, no fructifications were produced.

*Odontia hydnoidea* (figs. 2, 13)

*Odontia hydnoidea* (Cooke & Massee) v. Höhn.—K. Akad. Wiss. Wien.

Math. Naturw. Kl. Sitzungsber. Vol. 118: Abt. 1. p. 5-6. 1909.

Not *Odontia hydnoidea* (Schw.) Peck, —N.Y. State Mus. Bull.

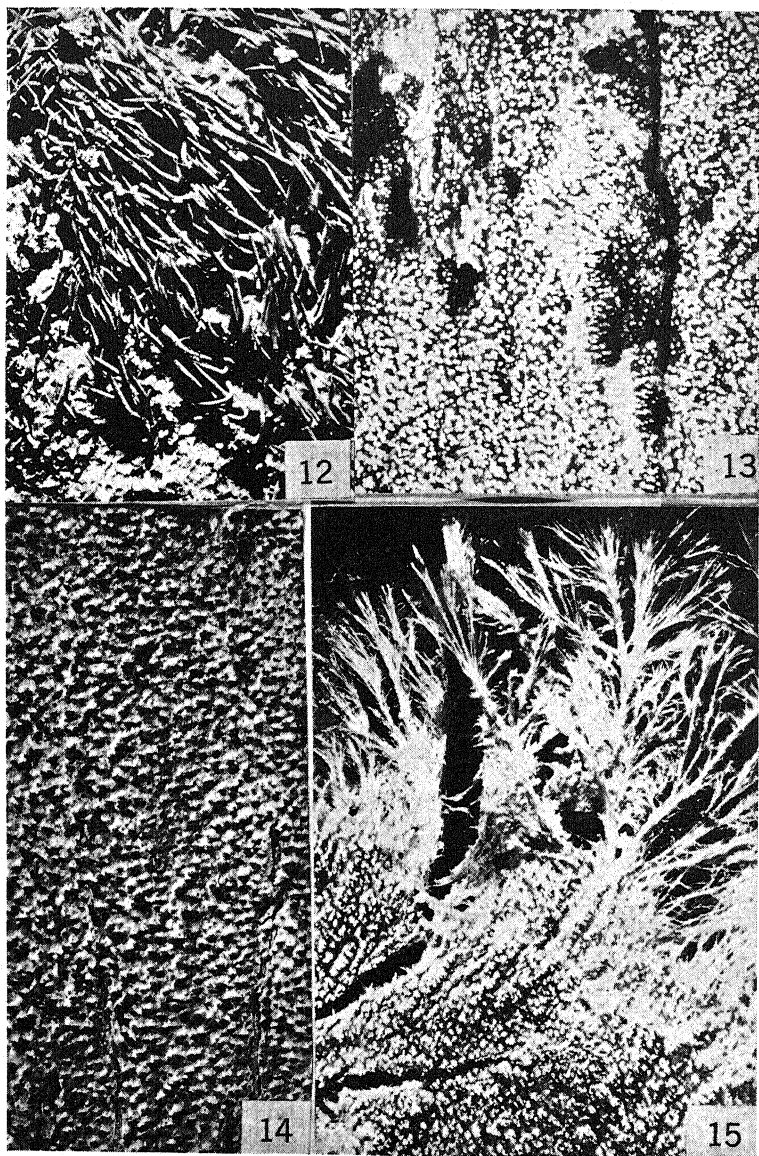
67. 39. 1903.

Fructification effused, in patches up to  $40 \times 15-25$  cm., thin, adherent, waxy, crustaceous, pale gull grey (R) to pale smoke grey (R) with wax yellow (R) areas, finally at maturity light buff (R) or chamois (R); margin subdeterminate; spines concolorous varying from mere granules to teeth averaging from 0.5 to 1 mm. in length, subulate, fimbriate, with rigid bristles scattered or in fascicles as seen under the hand lens, fragile, easily rubbed from the subiculum; hyphae of the subiculum compact, agglutinated, indistinct; cystidia abundant, strongly incrustated with oxalate, some with thick walls and narrow lumen, others with thinner walls and larger lumen, conical to fusiform,  $53-100 \times 10-21 \mu$ , layered in the subiculum, partially imbedded in the spines or projecting at various angles and fascicled at the apices; hyphae in axes of the spines incrustated, septate, cylindrical,  $8-12 \mu$  in diameter, extending beyond the apices  $20-40 \mu$ , thinner walled than the cystidia; basidia  $3.5-4 \mu$  in diameter, length not determinable; spores  $3-5 \times 1.5-2 \mu$ , oblong cylindrical, slightly flattened on one side, hyaline, usually with two guttulae. Incrustation soluble in hydrochloric acid.

The axillary incrustated hyphae have the power of regeneration. These hyphae, which are  $10-12 \mu$  in diameter, may from their apices give rise to hyphae  $4-5 \mu$  in diameter.

Specimens from England, Germany, France, and Holland were kindly loaned by DONK. A study of these, in comparison with 20 collections from Michigan and one from Louisiana, has convinced the writer that the American material is not specifically distinct from the European plants. The cystidia figured by VON HÖHNEL are very similar to those found in American collections.

In 1907 VON HÖHNEL and LITSCHAUER (21) described this fungus as a new species, *Peniophora crystallina*. In 1908 they (22) decided that this material was a delicate corticium-like variety of *Odontia conspersa* Bres., and reduced it to a variety, *O. conspersa* var. *crystal-*



FIGS. 12-15.—Fig. 12, *O. separans*: dried specimen collected by D. H. BELL, 1914. Fig. 13, *O. hydnooides* (no. 523) on rotten hardwood, Whitmore Lake, Mich. Gull grey (R) phase. Fig. 14, *O. bicolor* (no. 30) on coniferous wood, Nelson, N.Y. Determined by BOURDOT. Note darker tips to spines of dried specimen. Photograph by EDGERTON. Fig. 15, *O. fimbriata* (no. 427), Ann Arbor, Mich. Note fimbriate margin and exceedingly small, fimbriate spines.  $\times 5$ .

*lina* (v. Höhn. & Litsch.). In 1909 VON HÖHNEL (19) called attention to three prevailing forms of this fungus, the odontia-like, the grandinia-like, and the peniophora-like forms. At the same time he reported on his study of COOKE and MASSEE's original specimen of *Peniophora hydnoidea* which is an *Odontia*, and therefore created the combination *O. hydnoidea* (Cke. & Mass.) v. Höhn. That this fungus is decidedly variable can be seen from the treatment given it by different students. Thus VON HÖHNEL (21) and DONK (16) consider it a *Peniophora*, BRINKMAN (in VON HÖHNEL and LITSCHAUER 22) a *Grandinia* BOURDOT & GALZIN (5), and CEJP (13, 14) an *Odontia*. The writer believes that it is an *Odontia* because of the well developed spines present in the mature fungus.

The color of this fungus is variable. Some specimens are gull grey (R) and others are light buff (R) to chamois (R). The gull grey specimens are young forms. The color change from gull grey to whitish or light buff was observed for two collections. Both colors have been noted on the same specimen in several instances. The average American collection shows some minor variation from the European material, in that the teeth are better developed, the cystidia have thicker walls and are stouter. There are gradations in the shape and size of the cystidia between collections of the American and of the European specimens.

The monosporous cultures of this fungus produced a thin, skinlike layer of mycelium over the surface of the agar. Around the margin of the petri dish and on the drier areas of the media in the test-tubes, thick, white, appressed, felty mycelium formed. The submerged hyphae were thin walled, 2-5  $\mu$  in diameter, and were slightly incrustated with small granules, becoming agglutinated into bundles but not forming rhizomorphs. Clamp connections were present but rare. Globose chlamydospores were abundant. They were 8-16  $\mu$  in diameter, thin walled at first, becoming thick walled in old cultures. They stained very dark brown with iodine solution or chloral hydrate with iodine.

The presence of clamp connections and the fruiting of monosporous cultures demonstrated the homothallic nature of this fungus.

It is slow to fruit in culture, although it is stimulated by a reduction in food supply. When KOTILA's nutrient solution was replaced

by sterile distilled water in 10-day-old cultures on filter paper pads, fructification followed readily in 10-15 days. Check cultures were kept for 49 days with no signs of fruiting. When the nutrient solution was removed from these and replaced by sterile distilled water, basidia and basidiospores were produced in 11 days. The hymenium developed in culture had an irregular, crustlike formation and the typical, incrustated, fusoid cystidia characteristic of the field collections were not produced in culture in any case.

*Odontia bicolor* (fig. 14)

*Odontia bicolor* (A. & S.) Bres.,-Ann. Myc. 1:87. 1903.

*H. balsameum* Peck,-N.Y. State Mus. Bull. 75. p. 15. 1904.

*H. serratum* Peck,-N.Y. State Mus. Rept. 50. p. 112-113. 1897.

Fructification broadly effused, variable, 8-60×5-20 cm., thin, adnate, subtomentose and pruinose to waxy and pruinose, white when fresh to milk-white (R) to cream color (R) when dry, cracking; margin determinate; spines conical to subulate, short, 0.5-1.5 mm. long, solitary or crowded, apices fimbriate, serrate on sides, on drying often forming fasciculate clusters especially when the teeth are long, apices often rufescent when dry; hyphae of the subiculum compact, interwoven, 1.5-2  $\mu$  in diameter, septate with clamp connections; hyphae in the axes of the spines thick walled, 5-8  $\mu$  in diameter, often agglutinated and slightly yellowish, extending beyond the apices of the spines 20-50  $\mu$ , 3-5  $\mu$  in diameter and thinner walled; basidia clavate, 10-20×3-5  $\mu$ , 2-4 sterigmata 4-5  $\mu$  long; spores oblong ovoid, apiculate, hyaline, smooth, 4-6 (8) ×3-4  $\mu$ ; cystidia of two types: one globose, spiny oxalate crystals 8-16  $\mu$  in diameter, incrustated on hyphae 1.5-2.5  $\mu$  in diameter, occurring on the sides and apices of the spines or in the subiculum near the substratum, incrustations soluble in hydrochloric acid; the other capitate cystidia, incrustations 7-9  $\mu$  in diameter on the sides and apices of the spines, cap part soluble in potassium hydroxide, alcohol, and chloral hydrate with iodine.

Specimens from New York, Michigan, Louisiana, Jamaica, and Holland were studied. The New York collection (no. 30) was determined by BOURDOT.

The types of *Hydnum balsameum* and *H. serratum* in the Herbari-

um of the New York State Museum, Albany, New York, show the same spiny crystals and capitate cystidia which are so characteristic of this species.

Not all attempts to get this fungus into culture were successful. Good spore deposits were secured in every case, but the spores would not germinate readily in distilled water or on agar at room temperature (20° C.). Twenty germinated single spores were isolated from material collected in Louisiana (no. 342) but only three (nos. 1-3) grew. The growth habit of no. 3 was markedly different from that of nos. 1 and 2, as the mycelial mat formed was from three to four times the size of the others. The mycelium from the no. 3 culture was more superficial, byssoid, and zonate whereas in the other two it was more floccose and azonate. These single spore cultures did not produce any clamp connections on the hyphae after a period of five months. The hyphae were moderately thick walled, 2-3.5  $\mu$  in diameter, uniform in size, with occasional irregular swellings, and were septate. No chlamydospores were formed. The mycelia of these were mated as follows: 1 $\times$ 3, 1 $\times$ 2, and 2 $\times$ 3. Clamp connections were formed in the case of 1 $\times$ 3. From these results it would seem that this species is heterothallic.

This fungus is exceedingly slow growing in culture and forms a mycelium mat of 3-5 cm. in diameter in a period of three months. Small, hyaline, rod-shaped segments 3-5 $\times$ 2-2.5  $\mu$  occurred in the single spore cultures, but when the aerial mycelium of the culture was examined under the 8 mm. lens, hyphae made up of many short segments were found, similar to what BREFELD illustrated as oidia. When water was added to the mount, these segmented hyphae disappeared and short, rodlike segments were abundant.

*Odontia fragilissima* (figs. 18, 19)

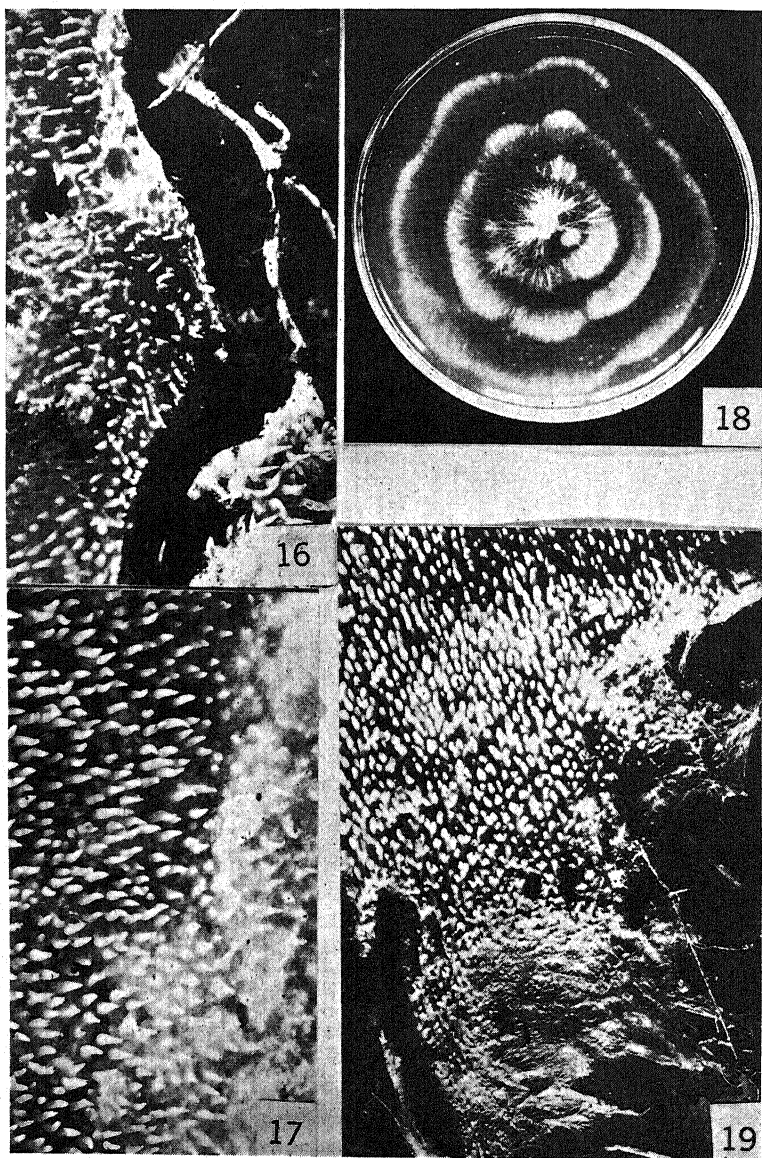
*Odontia fragilissima* (B. & C.) comb. nov.

*Hydnum chrysocomum* Underw.,-Bull. Torr. Bot. Club 24:82. 1897.

*Oxydontia fragilissima* (B. & C.) Miller,-Mycologia 25:294. 1933.

Fructification effused, 2-6 to 60 $\times$ 15 cm., thin, fleshy-membranaceous when fresh, papery-membranaceous when dry, separable from the substratum, yellow to chrome orange when fresh; margin white,





FIGS. 16-19.—Fig. 16, *O. himantia* showing rhizomorph. Fig. 17, *O. himantia* (no. 545) showing acute and obtuse spines and cottony subiculum. Ann Arbor. Fig. 18, petri dish culture of *O. fragilissima* grown at 25° C. Note concentric zonations. Fig. 19, *O. fragilissima* (no. 429), Ann Arbor, Mich. Note spines and rhizomorphs.  $\times 5$ .

byssoïd, fibrous, usually with orange (R) to chrome orange (R) rhizomorphs; spines subulate, for the most part concolorous with the hymenium, apex usually lighter colored, 1-3 mm. long, some slightly flat and connate at the base, distant to crowded, much slimmer when dry than when fresh; hyphae of the subiculum 6-9  $\mu$  in diameter, incrustated with fine, orange colored granules, thick walled, compact, clamp connections present but rare; basidia 14-16 $\times$ 4-5  $\mu$  in diameter, 4-spored, very compact, arranged on the sides and between the teeth; spores oblong, hyaline, 3-5 $\times$ 2-2.5  $\mu$ ; cystidia, when present, finely incrustated, 3-6  $\mu$  in diameter, moderately thick walled and projecting 10-20  $\mu$ ; lumps of oxalate present in the axes of the spines; hyphae in axes of the spines agglutinated forming a compact bundle, some with very fine incrustations.

The following collections were studied: the co-type of UNDERWOOD's species *Hydnum chrysocomum* in the Herbarium of the New York State Museum, also one other collection from New York, three collections from Michigan, three from Louisiana, one from Ohio, and one from North Carolina.

This plant can readily be recognized by its bright chrome-orange color, colored rhizomorphs, and the fragile, papery-membranaceous consistency of the dried specimens.

LLOYD (29) emphatically states that this species is *Sistotrema crocea* Schw., although he was unable to find any specimen of it in SCHWEINITZ' herbarium. It is difficult to see how LLOYD could mistake this well marked plant which is characterized by a thin, fragile, separable subiculum and colored rhizomorphs for a plant which was described as firm, tuberculose, rimose, and adnate.

BANKER (1) used the name *Hericium crocea* (Schw.) for plants which are parasitic on apple trees in North America and in Europe. The apple tree parasite is also known as *Hydnum schiedermayeri* Heuf., *Acia setosa* (Pers.) B. & G., and *Mycoacia setosa* (Pers.) Donk. DONK (16), however, believes that SCHWEINITZ' description does not refer to the apple tree parasite and that BANKER's use of SCHWEINITZ' name for this fungus is unfortunate.

MILLER (33) placed UNDERWOOD's name *Hydnum chrysocomum* in the synonymy of *Oxydontia fragilissima* but the writer believes the

plant belongs to the genus *Odontia* and has made the combination *Odontia fragilissima*.

A Louisiana collection (no. 332 from Pride, La.) consisted of a yellow colored specimen growing intermingled with an orange colored specimen on the same log and could not be distinguished from it other than by its color, as the microscopic details, presence of rhizomorphs, and consistency of the fruit bodies were identical. This yellow phase has also been noted by J. N. COUCH. With a specimen from Chapel Hill, North Carolina, he sent the following note: "This specimen varies from yellow to red, usually with orange rhizomorphs." In a later collection (no. 429) the chrome-orange fruit body was cut off from the wood and the bare stick placed in a moist chamber. In a few days it was covered with a whitish mycelium which soon became chrome-orange. After three to four weeks the color changed to capucine yellow (R), hence the yellow and orange phases were definitely connected.

A tissue culture from one of the Michigan collections was secured from rhizomorphs. It first produced a white, fibrous, aerial mycelium with hyphae 6-8  $\mu$  in diameter which had clamp connections. The mycelium was at first hyaline, then chrome-orange. The color is due in part to the fine granular incrustations which sheathe the hyphae, and in part to color in the protoplasm. The color is partly bleached out of the mycelium by potassium hydroxide, Amann's mounting medium, or chloral hydrate with iodine. The granules are somewhat loosened by these reagents; however, hydrochloric acid does not appear to attack them. Chloral hydrate with iodine stains the hyphae dark brown. Cultures grown in light on agar in flasks at room temperature produced concentric zonations around the point of inoculation, while cultures grown in darkness produced a fibrous aerial mycelium with abundant rhizomorphs. Petri dish cultures kept in the dark at 25°, 25°, and 30° C. produced concentric zonations similar to those formed in light (fig. 18). Here the zonation was broader and consisted of rings of aerial mycelium alternating with the submerged mycelium. All attempts to stimulate this fungus to fruit, such as reducing the food supply, changing the carbohydrate-nitrogen ratio, growing it at different constant temperatures and alternating high and low temperatures, failed.

Sixteen monosporous cultures were secured from no. 429, a Michigan collection. The primary mycelium consisted mostly of submerged hyphae of irregular size with swellings and contortions. About three weeks after isolation, the mycelium produced clamp connections, thus indicating a homothallic condition for this fungus. The secondary mycelium was more regular in diameter than the primary mycelium, and did not have the anastomosing network of branches found in the primary mycelium. The mycelium of the single spore cultures behaved like the mycelial cultures from the rhizomorphs in that after six months neither basidia nor basidiospores were formed.

*Odontia separans* (fig. 12)

*Odontia separans* (Peck) comb. nov.

*Hydnum separans* Peck, -N.Y. State Mus. Rept. 50. p. 112. 1897.

*Oxydontia macrodon* (Fr.) Miller, -Mycologia 25:294. 1933.

PECK described this species as follows:

Resupinate, white; subiculum membranous, at first pure white, becoming yellowish or cream color with age; aculei subulate, glabrous, crowded, 2-3 lines long, fragile, easily separating from the subiculum and leaving in it alveolar impressions; spores globose, colorless, 0.00016 inch broad.

Much decayed wood of deciduous trees. Adirondack Mountains. July.

After the teeth have been separated from the subiculum, it resembles somewhat a shallow-pored species of *Poria*. By this character, the thinner subiculum and the smaller spores the species may be separated from *H. mucidum*, to which it is allied.

PECK reported the spores as globose, colorless, 0.00016 inch broad (4.06  $\mu$ ). Examination of the type specimen in the Herbarium of the New York State Museum indicated that the spores are broadly ellipsoid, amyloid, and that two types of gloecystidia are present. The alveolar impressions which PECK noted are common also on other species of *Odontia* when the teeth break away from the subiculum.

MILLER (33) has placed PECK's name *Hydnum separans* in the synonymy of *Oxydontia macrodon* (Fr.) Miller (*Hydnum macrodon* Fr.). This treatment seems unwarranted because the Friesian de-

scription indicates a plant with almost no subiculum, whereas PECK's plant has a well developed subiculum. For this reason PECK's name is retained here.

Fructification effused, at first small, orbicular patches, 2 cm. in diameter, later up to  $7 \times 4$  cm., soft, membranaceous, separable, white when fresh, colonial buff (MP) to amber yellow (MP) when dry; margin indeterminate; spines up to 6 mm. long, subulate, crowded, fragile, easily separated from the subiculum leaving small pits; subiculum moderately dense, hyphae of two kinds, one moderately thick walled,  $2.5-4 \mu$  in diameter, often collapsing, septate and with clamp connections, the other very thick walled,  $2 \mu$  in diameter, walls so thick and lumen so narrow that they sometimes appear like capillary hyphae; basidia cylindrical to clavate,  $16-24 \times 7-10 \mu$ ; sterigmata  $3-5 \mu$  long; spores smooth, hyaline, broadly ellipsoid, amyloid,  $3.5-5 \times 3-4 \mu$ ; gloeocystidia of two types: one fusoid with acute to acuminate apices, the other in the axes of the spines thin walled, flexuous,  $125-150 \mu$  long,  $7-10 \mu$  wide, staining dark brown by chloral hydrate with iodine and reddish by potassium carbonate with eosin; spores turning blue under the action of iodine.

Two collections besides the type were studied, one from the vicinity of Ann Arbor, Mich., and one from Hemlock Ravine (? New Richmond, Mich.), collected by D. H. BELL, 1914, and in the Herbarium of the University of Michigan (fig. 12). The above description is based on these three collections.

Eight monosporous cultures and one polysporous culture of this species were made. The mycelia of the monosporous cultures did not have clamp connections. The mycelium of the polysporous culture showed clamp connections. Therefore, the fungus is apparently heterothallic. It has not fruited in the two years it has been in culture. The mycelia of the single spore cultures have hyphae of two sizes, one  $1.5 \mu$  in diameter, thick walled, the other  $2.5-3 \mu$  in diameter, thick walled, septate. Thick walled, intercalary chlamydo-spores are produced on hyphae of monosporous cultures. These vary from ellipsoid,  $4-6 \times 2-5 \mu$ , to globose up to  $8 \mu$  in diameter. Some granular oxalate incrustations were produced on the hyphae. No gloeocystidia were produced in culture.

*Odontia himantia* (figs. 16, 17)

*Odontia himantia* (Schw.) Bres.,—Ann. Myc. 1:84. 1903.

*Hydnum subfuscum* Peck,—N.Y. State Mus. Rept. 40. p. 55. 1885.

Fructification effused, size irregular depending upon the substratum, cottony tomentose, thin, at first white, then antique gold (MP) to fuscous when old, separable when fresh, sometimes slightly adherent; margin byssoid, determinate, usually with conspicuous rhizomorphs; spines up to 4–5 mm. long, scattered or gregarious, slender, obtuse or conical (often drying conical), entire, white when fresh to fuscous; hyphae of subiculum 2.5–3.5  $\mu$  in diameter, smooth, or minutely roughened, septa and clamp connections present; axillary hyphae mostly thin walled, 2.5–4  $\mu$  in diameter, agglutinate; basidia clavate, 2–4 spored, 18–38  $\times$  5–8  $\mu$  in diameter; sterigmata subulate, straight, 6–7  $\mu$  long; spores cylindrical, often apiculate, hyaline, smooth, size variable depending upon maturity, 8–12 (14)  $\times$  3–5  $\mu$ ; no cystidia.

Common on old, rotten frondose or coniferous wood, especially oak. Mycelium of this species appears in the soil and on débris in mid-summer. Fruiting specimens can be found as early as the middle of August, although the best development is later in the fall.

An examination of the type of *Hydnum subfuscum* Peck confirmed KAUFFMAN'S (25) belief that PECK'S species is the same as *Odontia himantia*. PECK commented on the similarity of *H. subfuscum* to *H. himantia* but believed that the acuteness of the spines in *H. subfuscum* denoted a specific difference. The occurrence of obtuse and acute spines on the same plant has been noted and moreover the obtuse spines often become acute on drying.

BOURDOT and GALZIN (5) have placed this species in the genus *Clavaria* under the section *Ceratella*. However, the hymenium occurs between and continuous with the spines and thus it properly belongs in the *Hydnaceae*.

*Odontia fimbriata* (fig. 15)

*Odontia fimbriata* Fr.,—Genera Hymenomycetum 13. 1836.

*Mycoleptodon fimbriatum* (Fr.) B. & G.,—Bull. Soc. Myc. France 30:276. 1914.

*Gloiodon fimbriatum* (Fr.) Donk.—Ned. Bot. Ver. 1:79. 1930.

*Ettheiroduon fimbriatum* (Fr.) Banker, -Bull. Torr. Bot. Club  
29:441. 1902.

Fructification effused, size variable, 8-20×5-10 cm., or in small patches, thin, membranaceous when fresh to papery or slightly coriaceous when dry, separable; nude (MP) to onion-skin pink (MP); margin fimbriate and with conspicuous rhizomorphs; spines small, short, conical, crowded, apices appearing bristly under a handlens; hyphae of the subiculum of two kinds, one moderately thick walled, rarely septate, 2-4  $\mu$  in diameter, the other thin walled, 3-5  $\mu$  in diameter, septations frequent, clamp connections of the same diameter as the hyphae; basidia cylindrical, clavate, 13-18×2.5-3.5  $\mu$ ; spores oblong-ovoid, smooth, hyaline, 3-4×2  $\mu$ ; cystidia cylindrical, thick walled, septate, 6-19  $\mu$  in diameter, projecting beyond the spines 10-50  $\mu$ , heavily incrustated with oxalate, soluble in hydrochloric acid. (Potassium hydroxide aids in distinguishing these better than do other reagents.)

Widely distributed on rotten wood. Specimens from Michigan, Pennsylvania, New York, and Ohio were studied.

BOURDOT and GALZIN (4) transferred this species to the genus *Mycoleptodon* because they consider the plant coriaceous and because it has thick walled hyphae making up the subiculum and cystidia similar to those in *M. ochraceum* (Pers.) B. & G. It is not so coriaceous as *M. ochraceum*, however, nor has it ever been found to be effuso-reflexed or dimidiate as are other species included in *Mycoleptodon*, and therefore should be placed in *Odontia*. There are other species of *Odontia* with thick walled, flexuous hyphae and cystidia similar to those in *O. fimbriata*.

*Odontia setigera*

*Odontia setigera* (Fr.) Miller, -Mycologia 26:19. 1934.

*Kneiffia setigera* Fr., -Genera Hymenomycetum 17. 1836.

*Kneiffia setigera* Fr. Bresadola, -Ann. Myc. 1:103. 1903.

*Odontia vesiculosa* Povah non Burt., -POVAH, Papers Mich. Acad. Sci., Arts, Letters 9:262. 1929.

Fructification effused, in small patches 1-2 cm. on a side, coalescing to form areas 15-30×8-20 cm., separable when fresh and slightly so when dry, very much cracked when dry, whitish when

fresh, agate grey (MP) to ivory (MP) when dry, finally becoming buff (MP) in the herbarium; margin arachnoid, pubescent, subdeterminate to determinate; spines variable, hemispherical to obtuse or cylindrical, less than 0.5 mm. long; hyphae of the subiculum loosely interwoven next to the substratum, thin walled, 3–5  $\mu$  in diameter; basidia 16–24  $\times$  4–6  $\mu$ ; sterigmata 4–6  $\mu$  long; spores oblong-cylindrical, hyaline, 6–12  $\times$  3–4  $\mu$ ; cystidia imbedded in the axes of the spines and projecting 30–80  $\mu$ , 6–10  $\mu$  in diameter; irregular, incrustated with oxalate crystals; cystidia, under the handlens appearing as shining "setae," thin walled, septate, clamp connections on the septa revealed after the incrustation has been dissolved in hydrochloric acid.

On rotten wood, Michigan, Iowa, New York, Louisiana.

BURT gave several herbarium names to this fungus, one of which was *Odontia vesiculosa* based upon a collection by A. H. ПОВАН from Vermilion, Michigan. ПОВАН (37) published the name "*Odontia vesiculosa* Burt, ined.," and gave the host and spore measurements. An examination of his collection in the Herbarium of the University of Michigan has shown that it is *Odontia setigera*.

### Discussion

One of the outstanding results of this study is the discovery that a majority of the species are homothallic. BLAKESLEE (2) first reported the occurrence of heterothallism in fungi among the Phycomycetes. Later this phenomenon was found in the Ascomycetes and Basidiomycetes. In the latter, heterothallic forms have been demonstrated among the Uredinales, the Ustilaginales, and the Agaricales. Only a few species of the Basidiomycetes which have been investigated have been homothallic. GÄUMAN and DODGE (18), in discussing sexuality of the Basidiomycetes, state "true homothallism is rare" although "it has been experimentally demonstrated" in certain agarics.

*Odontia fusco-atra*, *O. stenodon*, *O. uda*, *O. arguta*, *O. hydroides*, and *O. fragilissima* all proved to be homothallic. Strains from single basidiospores produced cultures which had clamp connections on the mycelium and these cultures formed basidia and basidiospores. *O. bicolor* and *O. separans* apparently are heterothallic species. Strains from single basidiospores remained sterile, and the mycelia,



when mated in certain combinations, produced clamp connections. A sufficient number of monosporous cultures were not obtained to justify conclusions covering sexuality.

As was previously mentioned, microconidia, macroconidia, chlamydospores, bulbils, and oidia have been reported by various investigators of these fungi. In cultures of the homothallic species, no bulbils, microconidia, macroconidia, nor oidia were observed. Chlamydospores were present in cultures of both homothallic and heterothallic species. *Odontia bicolor* produced oidia on the mycelium derived from a single spore. Examinations of European and American collections of *Hydnum erinaceus* Bull. and *H. coralloides* (Scop.) Fr. failed to show the macroconidia or microconidia reported by DE SEYNES or by PATOUILLARD. The grapelike clusters of spores which they interpreted as conidia were seen, but when potassium hydroxide was added to the mount, the spores separated and a bare hypha was revealed, around which the spores were clustered, and this could not be interpreted as a conidiophore. When iodine was added, these spores turned blue, as did the spores attached to the basidia. The walls of the basidiospores of these species are viscid, as shown by the pronounced adherence of the spores to each other and to the hymenium. It seems probable that the microconidia and macroconidia which DE SEYNES and PATOUILLARD described are in reality basidiospores adhering in clusters.

The artificial environment of these fungi in pure culture on different types of media had a marked effect upon the fructification produced. Only two species produced fructifications similar to those formed in nature; namely, *Odontia arguta* and *O. stenodon*. These produced typical spines on nutrient agar, on filter paper pads in nutrient solution, and on woods wet with different nutrient solutions. All of the other species which fruited in culture produced granular crustlike fructifications with no regularity. Thus one of the diagnostic characters of the genus *Odontia*, the presence of a hymenium upon spines, may be decidedly modified or eliminated in artificial culture.

The external appearance of the fructification of *Odontia arguta* in culture was similar to that in nature. However, no characteristic cystidia were developed in culture. The formation of cystidia could

not be induced in cultures of different ages by varying the conditions of heat, light, moisture, and substratum. This lack of cystidia was noted in all cultures, with the exception perhaps of *O. fusco-atra*. This fungus produced scattered, individual, incrusting hyphae (or cystidia?) which projected beyond the hymenial layer. There was no tendency to form a spine around these hyphae, as occurs in nature.

The mycelium of *Odontia uda* in culture did not turn violet color when exposed to the fumes of ammonia as did field collections, and the anise odor of fresh field collections could not be detected in the cultures.

The classification of the resupinate species of the Hydnaceae has undergone various changes with the emphasis that different investigators have placed on the diagnostic characters such as consistency of the fructification, presence or absence of cystidia or other sterile organs, character of the spines, and color of the spores.

The consistency characters of the fructification such as fleshy, waxy, membranaceous, crustaceous, and coriaceous are rather difficult to interpret in these fungi. In fact several students, in describing the same species, have ascribed different consistencies to it. The use of consistency characters for delimiting genera seems to be of doubtful value.

The presence of cystidia is used extensively as a diagnostic character in the classification of fungi. In the Agaricaceae and the Polyporaceae, however, the occurrence of cystidia is not considered of sufficient value to separate genera. BURT (8), in his work with the Thelephoraceae, used the presence of cystidia to separate *Peniophora* from *Corticium*, but did not consider the presence of gloecystidia a generic character. An example of the artificial classification which has resulted from the use of the occurrence of cystidia to separate genera is afforded by the genera *Mycoleptodon* and *Pleurodon*. BOURDOT and GALZIN (4, 5) used the presence of cystidia as one of the characters in *Mycoleptodon* to separate it from *Pleurodon*. In 1932 BOURDOT (3) directed attention to the fact that cystidia occur in one species of *Pleurodon* and are absent in several species of *Mycoleptodon*. He therefore united these two genera. Also the distribution of cystidia in a specimen is often variable, occurring in some portions

and not in others. The use of such a variable character to delimit genera results in an artificial classification and tends to separate closely related species.

The aculei vary from mere hemispherical granules to large subulate spines. They are scattered or gregarious on the subiculum, and often slightly confluent at their bases, especially where the spines are crowded or where the fructification grew on the side of a log. The apices of the spines may be obtuse to acute, or fimbriate or dentate. Drying of specimens causes a distortion of the spines and often a fasciculate clumping. The tendency has been to use the clumping of the spines, their coalescence, and the types of the apices as generic characters. For example, *Odontia* has been characterized by fimbriate or penicillate apices to the spines and *Acia* by entire tips to the spines. However, rejuvenation of the hyphae in the spine following a period unfavorable for growth will cause a fimbriate appearance to an otherwise entire spine. The size and character of the spines vary with the age of the specimen. The fasciculate clumping or molariform type of tooth results from drying in most instances.

The echinulate spore has also been used to delimit genera. In this group, however, there are only a few species with hyaline echinulate spores, and in most instances the markings are not readily visible. The use of this character for generic distinction in the Hydnaceae would result in the establishment of new genera with the same morphological characters as the existing genera, with the exception of the spore marking.

The amyloid condition of the spore has also been used as a generic separation. It also separates otherwise closely related species.

The classification of BOURDOT and GALZIN (5) was chosen as the best treatment to follow when these studies were started in 1929. However, the results obtained did not justify following this classification completely. They recognize four genera of the "Hydnés" as being always resupinate: *Radulum*, *Grandinia*, *Acia*, and *Odontia*. As has been indicated, these genera are differentiated by characteristics that separate closely related species. DONK (16) also considers that these genera are artificial and should be combined. As a result of these studies, the following emendation of the genus *Odontia* is proposed:

*Odontia* Fr., emended.

Synonyms: *Mycoacia* Donk; *Acia* Karst.; *Grandinia* Fr.; *Gausapia* Fr. pro parte; *Radulum* Fr. pro parte; *Kneiffia* Fr. pro parte; *Oxydontia* Miller.

Receptacle resupinate; consistency varied, membranaceous, crustaceous, waxy, arachnoid, soft or floccose; covered with granules, persistent, hemispherical, or with spines, conical or irregular, apices entire or penicillate; cystidia, gloeocystidia, and cystidioles present or absent, incrustated or naked; basidia 2-4-6-8 spored; spores hyaline, smooth or minutely echinulate, amyloid or non-amyloid. The type species is *Odontia fimbriata* Fr.

The type of the genus *Radulum*, *Radulum aterrinum* Fr., is an Ascomycete belonging to the genus *Eutypa* according to VON HÖHNEL (20). Many of the species since placed in the genus *Radulum* are typical odontias.

The type of the genus *Kneiffia*, *Kneiffia setigera* Fr., has been placed in the Thelephoraceae and in the Hydnaceae by different authorities, but is, in its best stage of development, a true odontia according to MILLER (34), who has transferred it to this genus.

The genus *Grandinia* Fr. is usually characterized by the hemispherical granules and by the absence of cystidia. As heretofore indicated, the shape and size of the spines and the absence of cystidia are characters too variable to be used for generic delimitation. Those who recognize the genus *Grandinia* admit that its separation from *Odontia* is artificial. The name *Grandinia* is antedated by *Gausapia* Fr., which, from its use in the Genera Hymenomycetum, should be the valid name if the segregation were maintained.

The genus *Acia* Karst. is usually separated from *Odontia* by the waxy consistency of the fructification and by the entire apices to the spines. Such a separation is artificial. Many of the species recognized in *Odontia* are described as waxy. The entire tip to the spine is very variable, even in the same fructification.

The name *Acia* is also not valid, as it has been used previously for a genus of the Rosaceae. DONK (16) proposed the name *Mycoacia* as a substitute for the invalid name *Acia*. Recently MILLER (32) proposed a new genus *Oxydontia* with the type *O. setosa* (Pers.) Miller.

The generic description, together with the species included in the genus, indicates that instead of a new genus a new name has been proposed for the non-valid name *Acia*.

### Summary

1. In studying species of *Odontia*, the use of reagents such as chloal hydrate with iodine and potassium carbonate with eosin has aided in distinguishing structures like cystidia, gloeocystidia, and subhymenial hyphae. The differential action of these reagents on spores, gloeocystidia, cystidia, and other hymenial elements offers supplementary characters for the identification of species. Likewise a knowledge of the corrosive action of these reagents on these structures is important. It has been shown that variations in descriptions of different investigators can in some cases be traced to the difference in the reagents used in the study of specimens.

2. The necessity for revising the generic concept is shown by this study, and an emended description of the genus *Odontia* Fr. is given. This genus is emended so as to include the species formerly included in the genera *Mycoacia* (*Acia*, *Oxydontia*), *Grandinia*, *Radulum*, and *Kneiffia*.

3. Two new combinations are made, *Hydnum fragilissimum* B. & C. and *H. separans* Peck being transferred to the genus *Odontia*.

4. A study of PECK's types revealed that *Hydnum carbonarium* is the same as *Odontia fusco-atra*; *Hydnum serratum* and *H. balsameum* are the same as *O. bicolor*.

5. The following species were grown in pure culture for the first time: *O. fusco-atra*, *O. uda*, *O. bicolor*, *O. arguta*, *O. separans*, *O. fragilissima*, *O. hydnoides*, *O. stenodon*, and *O. brinkmanni*.

6. The following species produced basidia and basidiospores in culture: *O. fusco-atra*, *O. uda*, *O. arguta*, *O. hydnoides*, and *O. stenodon*.

7. Only two species, *O. arguta* and *O. stenodon*, produced fructifications similar to those produced in nature. Cystidia, an important diagnostic character, were not formed in culture.

8. The following seven species proved to be homothallic, mycelium from single spores developing clamp connections and producing

basidiospores: *O. fusco-atra*, *O. uda*, *O. arguta*, *O. fragilissima*, *O. hydroides*, and *O. stenodon*.

9. *O. bicolor* and *O. separans* proved to be heterothallic, the mycelium from monosporous cultures lacking clamp connections which were formed only when two compatible strains were mated.

The writer expresses his appreciation to those who aided in these studies: to the late C. H. KAUFFMAN under whose guidance the problem was started; to E. B. MAINS under whose direction the studies were completed; to H. D. HOUSE for the privilege of studying PECK's types; to ABBÉ H. BOURDOT and M. A. DONK for the numerous identifications and the loan of specimens; and to H. H. BARTLETT for editorial suggestions.

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# EFFECTS OF ENVIRONMENT UPON THE ROOT HABITS OF CERTAIN DECIDUOUS FOREST TREES<sup>1</sup>

HAROLD H. BISWELL

(WITH FOURTEEN FIGURES)

## Introduction

An experimental study of the development of roots and shoots of eight species of deciduous forest-tree seedlings was made near Fayette, Missouri, during 1933. Their growth in clay, alluvial, and loess soils was ascertained as well as the effects of shading and of deficient aeration. Root development of numerous saplings, 3 to 16 years of age, was also studied in different types of soil that occurred in relatively close proximity.

A study of the initial root habits of trees and their responses to changes in the external environment affords valuable criteria for judging the probability of the survival of a species when seeded on a particular soil type. It also leads to an understanding of natural reproduction as well as to an explanation of the course of succession. Since the area is part of a deciduous forest bordered by prairie, the findings are of particular interest in interpreting the relationship between forest and grassland.

Relatively little work has been done in America on the root habits of deciduous forest trees. CLEMENTS, WEAVER, and HANSON (6), working with seedlings of five deciduous species, found that the roots were greatly affected by different degrees of competition with grasses. AALTONEN (1) reached the conclusion that "the space arrangement of those parts of the trees which are above the soil is mainly decided by their roots and the competition existing between them for the water and food in the ground." BÜSGEN and MÜNCH (5), ROGERS and VYVYAN (19), and ADAMS (2) reached similar conclusions. Recently PARTRIDGE and VEATCH (17), SWEET (22), and YOCUM (29) in America, ROGERS and VYVYAN in England (19),

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KVARAZKHELIA (14) in Russia, working with fruit trees, and NUTMAN (16) in Africa, working with coffee, have found that the roots are greatly influenced by soil conditions. GURSKY (9) has shown that even related species of mature trees may differ in their root habits, not only as to penetration into the soil but also as to root type. WEAVER and KRAMER (27) state: "Although the root habits of a tree are governed, first of all, by the hereditary growth characters of the species, they are often quite as much the product of environment."

HAASIS (10) showed that soil type greatly modified root form of western yellow pine seedlings. HEYWARD (11) found that soil texture influenced considerably the extent of laterals of longleaf pine. ANDERSON and CHEYNEY (3) concluded that in evergreens the soil texture affects the length chiefly of the tap root. STEVENS (21) ascertained that the rate of growth of roots of white pine was due to a combination of environmental factors.

That light and aeration play an important rôle in shoot and root development has been shown by TOUMEY (23, 24), HOLCH (13), HOFFMAN and SCHLUBATIS (12), and DEAN (8). Excellent resumés of the literature regarding root habit have been given by BÜSGEN and MÜNCH (5), LAITAKARI (15), and STEVENS (21).

#### Material and methods

Seeds of the following trees were stratified out-of-doors in sand during the winter at a depth of 6 inches: *Gleditsia triacanthos* L., *Acer negundo* L., *Platanus occidentalis* L., *Juglans nigra* L., *Aesculus glabra* Willd., *Quercus maxima* (Marsh.) Ashe, *Hicoria ovata* (Mill.) Britton, and *Acer saccharum* Marsh. Early in March the seeds were planted in rows in cleared unshaded plots, one lot in upland clay, one in alluvial soil on a floodplain, and one in loess. When the seedlings began to appear one-half of each plot was shaded by lath frames. The frames were 2 feet high and covered on the top and south side with laths, each lath alternating with a space of similar width. For convenience, conditions under the frames will be designated as half shade. The seedlings were thinned so that they were 1.5-2 feet apart.

Growth in height was measured each two weeks. Number of

leaves, total leaf area, and dry weight of tops of representative samples were ascertained on July 1 and September 1. The form and extent of the root system of each species were determined in mid-summer and also in early fall. Rates of transpiration under the two conditions were measured at one upland station from twelve individuals of each of five species grown in sealed containers. Environmental factors were measured regularly throughout the entire growing season.

Studies were made on the form and extent of the roots of 35 saplings. In nearly all cases isolated specimens were chosen, hence the roots were free from those of other trees. Several trees of each species were excavated.

### Ecological factors

#### PRECIPITATION

The mean annual precipitation is sufficient to permit the growth of an oak-hickory forest, which, as a result of clearing and agricultural practices, has now almost disappeared. Mean annual precipitation during the last 20 years was 37 inches. The greatest yearly rainfall was 50.9 inches, the least 26 inches. Normally the rain is well distributed throughout the growing season. During 1933, 3.6 inches fell in March, 2.6 in April, 7.8 in May, 1.3 in June, 0.8 in July, and 2.6 in August.

#### SOIL

The soils are diverse in nature although the most distant stations were only 18 miles apart. Their chief characteristics are shown in table I.

The clay soil on the gentle northwest slope became very hard as it dried with the advance of summer. The alluvial soil was that of the floodplain of Moniteau Creek. It is flooded for a few weeks annually and, as shown by the mottling of the deeper layers, is poorly aerated. The station with loess soil was near the crest of a ridge only a mile from the Missouri River. The land sloped gently northward.

**WATER CONTENT.**—Soil samples were taken to a depth of 3 feet at all stations once each week from both the unshaded and shaded soil. The percentage of moisture was computed on the basis of oven-dry weight. Although the summer was dry, figure 1 shows that water

was always available for growth at all depths in the clay soil. In the unshaded loess soil the lowest recorded amount was 5.1 per cent in the surface 6 inches. With one exception, available water in the surface layer of alluvial soil always exceeded 10 per cent even under full insolation (table II). In fact, in the alluvial soil, water content was sometimes so high that it decreased aeration considerably.

TABLE I  
TEXTURE, ACIDITY, HYGROSCOPIC COEFFICIENT, AND MOISTURE  
EQUIVALENT OF SOILS AT SEVERAL STATIONS

SOIL, DEPTH IN INCHES	TEXTURE			pH	HYGRO- SCOPIC COEFFI- CIENT	MOISTURE EQUIVA- LENT
	CLAY	SILT	SAND			
Clay						
0-6.....	25.29	47.32	27.39	4.48	7.3	20.2
6-12.....	38.05	44.55	17.40	5.29	10.7	25.9
12-24.....	41.51	46.12	12.37	5.64	13.8	30.6
24-36.....	37.73	47.22	15.05	4.72	12.9	29.2
Alluvial						
0-6.....	29.78	57.17	13.05	5.17	7.7	26.6
6-12.....	32.30	45.97	21.73	5.35	8.1	25.8
12-24.....	32.35	45.51	22.14	4.80	8.3	24.5
24-36.....	35.03	47.09	17.88	4.99	10.3	26.6
Loess						
0-6.....	22.38	29.27	38.35	6.28	7.2	20.0
6-12.....	27.20	38.05	34.75	5.89	7.7	23.3
12-24.....	27.93	41.35	30.72	5.80	8.7	24.5
24-36.....	25.00	43.95	31.05	5.74	9.3	24.1

TEMPERATURES.—Temperatures of the soils were taken each week at various depths to 2 feet. They were obtained simultaneously in the open and shade at a station on sunny days. They were also measured hourly from 6 A.M. to 7 P.M. once each month. The results are summarized in table III. The loess soil was always slightly warmer than the clay or the alluvial soil, which usually had about the same temperature. The differences, however, were probably not great enough materially to affect the rate of growth in the different soil types.

#### LIGHT

Light intensity was measured at noon on clear days by means of a Clement's photometer. The average light intensity under the lath

shelters was 33 per cent of full sunshine. The percentage of sunshine for the summer was very high. April and May had 57 and 62 per cent clear days, respectively; June, 89; and July, August, and September, 85, 65, and 73 per cent respectively.

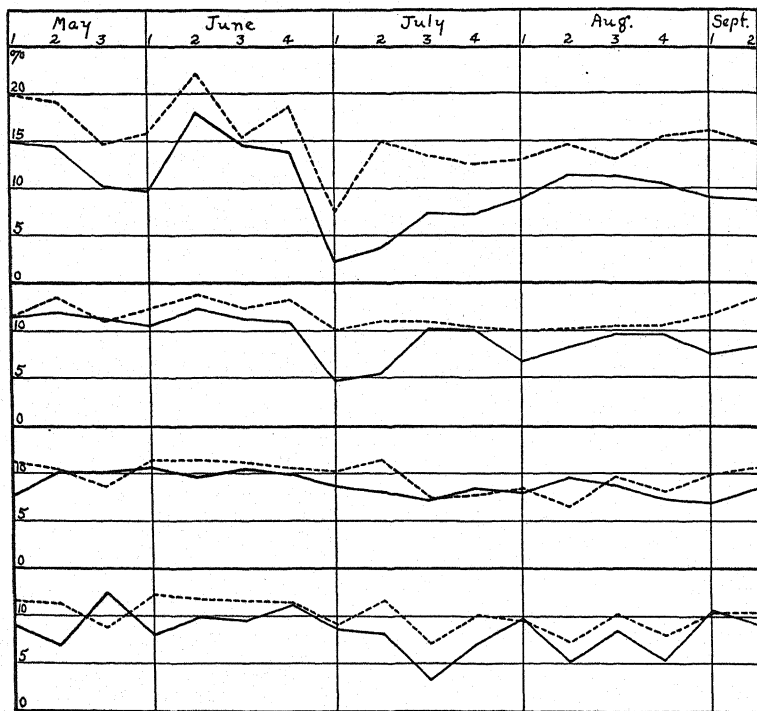


FIG. 1.—Water content in excess of hygroscopic coefficient in clay soil at depths of (upper lines) 0-6, 6-12, 12-24, and 24-36 inches. Solid line indicates full insolation, broken line half shade, and numbers the consecutive weeks of the months.

#### HUMIDITY AND AIR TEMPERATURE

Humidity under the lath shelters was often 7 per cent higher than in the open, but differences of 3-4 per cent were more usual. Differences in humidity at the several stations were slight. Temperatures varied even less. They were usually 2° to 3° F. higher under full insolation, but maximum differences of 6° F. were found. Maximum differences in both humidity and temperature occurred between

noon and 4 P.M. Average daily maximum humidities during May and June were about 80 per cent and during July and August about

TABLE II

WATER CONTENT IN EXCESS OF HYGROSCOPIC COEFFICIENT AT VARIOUS DEPTHS AT THREE STATIONS THROUGHOUT THE SUMMER

STATION AND AMOUNT	0-6 INCHES		6-12 INCHES		12-24 INCHES		24-36 INCHES	
	OPEN	SHADE	OPEN	SHADE	OPEN	SHADE	OPEN	SHADE
Clay								
Maximum....	17.8	22.1	12.3	13.7	10.9	11.8	12.4	12.0
Average.....	10.2	15.3	9.6	11.7	8.8	9.7	8.4	10.1
Minimum.....	2.1	7.6	5.5	10.0	7.1	6.4	3.5	7.0
Alluvial								
Maximum....	27.6	26.4	20.7	23.6	16.0	16.6	15.6	15.6
Average.....	16.0	17.5	12.7	16.2	13.2	13.9	12.1	12.5
Minimum.....	7.4	12.3	9.5	9.3	10.5	9.1	5.4	9.6
Loess								
Maximum....	15.7	18.9	16.5	16.6	15.4	16.0	16.3	16.4
Average.....	10.8	13.4	11.7	13.4	12.4	13.3	13.0	13.6
Minimum.....	5.1	7.7	7.6	9.5	10.0	9.0	10.3	10.3

TABLE III

AVERAGE AND EXTREME TEMPERATURES OF SOILS AT VARIOUS DEPTHS DURING THE SUMMER

SOILS AND TEMPERATURES	1 INCH		3 INCHES		12 INCHES		24 INCHES	
	OPEN	SHADE	OPEN	SHADE	OPEN	SHADE	OPEN	SHADE
Clay								
Maximum....	110.8	90.0	100.0	88.2	83.3	78.4	79.2	76.7
Average.....	98.1	83.3	90.5	79.4	77.4	73.3	73.5	70.4
Minimum.....	82.0	73.4	79.0	71.0	67.0	66.0	61.0	59.9
Alluvial								
Maximum....	111.3	89.6	101.2	87.8	83.2	80.5	82.0	78.8
Average.....	96.0	81.9	89.9	79.3	77.1	73.6	73.5	71.3
Minimum.....	77.0	68.0	76.2	67.6	66.0	64.4	61.0	60.0
Loess								
Maximum....	112.3	100.4	98.2	88.2	87.4	85.1	82.4	80.5
Average.....	99.4	84.2	90.6	79.8	78.1	74.2	75.1	72.1
Minimum.....	79.7	70.7	74.3	68.0	65.3	60.8	59.9	59.0

75 per cent. The corresponding average daily minimum humidities for the months just given were about 54 and 43 per cent.

The mean maximum daily temperature for the last two weeks in May was 79° F. This increased in June to 91.5° and in July to 92.9°. It averaged 87.7° during August. The mean minimum daily temperature for the last two weeks in May was 58°, and for the other months as follows: June 63.1°, July 65.4°, and August 62.2°.

#### WIND AND EVAPORATION

An average of 500 readings of wind movement, taken alternately in the open and under the lath shelters, gave a velocity of 609 feet per minute in the open but only 143 (77 per cent less) under the shelter. Average daily evaporation losses from Livingston's standardized, white spherical atmometers placed under full insolation and in the shade were (in cubic centimeters): May 28.9 and 20.7, June 41.4 and 31.9, July 49.1 and 38.2, August 29.4 and 22.7, September 23.5 and 15.7. For the entire summer the average in the open was 34.5 cc. per day and in the shade 25.8 cc. Greatest differences occurred on sunny days when the wind velocity was high.

#### Observations

The eight species previously listed, except *Hicoria ovata* and *Acer saccharum*, were grown in all three soil types. The *Hicoria* was grown in the clay and loess soils and *Acer* in the clay and alluvial soils. The soil was cultivated lightly. Ninety-one trees were excavated by the trench method during midsummer and the root habits of 93 additional seedlings were studied in the fall. Saplings excavated consisted of the eight species enumerated (except *Aesculus glabra*) and in addition *Quercus macrocarpa* Michx. and *Populus sargentii* Dode.

#### *Gleditsia triacanthos*

SEEDLINGS.—The seeds of honey locust germinated readily, but the seedlings grew slowly in early spring. Growth was rapid during the sunny humid days of summer. Table IV shows that early growth was greatest in the clay soil. Here the seedlings were 16 per cent taller than in the loess soil and 57 per cent taller than on the floodplain. Growth in sun and shade was the same in the clay but differences occurred in the other soils. A maximum growth of 1 inch per day was sometimes attained under full insolation during mid-

TABLE IV

HEIGHT IN INCHES OF SPECIES AT THREE STATIONS IN FULL INSOLATION  
AND IN PARTIAL SHADE

SOIL AND SPECIES	JUNE 1	JUNE 29	JULY 28	AUGUST 25	SEPTEMBER 7
Clay					
Honey locust.....	4.4 (4.4)*	7.5 (7.1)	9.7 (8.9)	11.4 (10.5)	11.4 (10.8)
Boxelder.....	4.2 (4.1)	5.9 (5.9)	11.7 (12.2)	16.2 (18.1)	16.4 (18.2)
Sycamore.....	0.8 (0.9)	2.0 (2.7)	5.4 (8.0)	11.0 (15.4)	11.0 (15.4)
Black walnut.....	7.7 (8.5)	9.0 (12.8)	9.2 (14.9)	9.2 (14.9)	9.2 (15.0)
Buckeye.....	1.8 (2.6)	1.8 (2.7)	2.4 (2.6)	2.9 (2.8)	2.9 (2.8)
Red oak.....	4.1 (4.5)	6.6 (7.5)	7.8 (11.3)	8.5 (11.3)	8.3 (11.6)
Shellbark hickory.....	4.2 (2.8)	4.6 (5.4)	4.6 (5.1)	4.3 (5.0)	4.3 (5.0)
Hard maple.....	2.0 (1.8)	1.7 (2.8)	2.4 (4.0)	2.2 (4.2)	2.2 (4.1)
Alluvial					
Honey locust.....	2.8 (3.1)	5.5 (4.6)	14.3 (8.8)	22.0 (11.6)	22.6 (12.1)
Boxelder.....	3.2 (4.1)	3.5 (4.5)	5.3 (5.9)	6.8 (6.7)	7.1 (6.9)
Sycamore.....	0.8 (0.8)	2.0 (1.6)	6.7 (4.0)	13.3 (10.0)	13.8 (10.0)
Black walnut.....	6.7 (6.7)	10.4 (10.7)	11.1 (12.6)	11.6 (12.5)	11.6 (12.5)
Buckeye.....	1.6 (1.3)	1.5 (1.2)	1.2 (1.4)	1.3 (1.5)	1.3 (1.5)
Red oak.....	3.5 (4.2)	3.5 (4.5)	9.0 (12.0)	9.8 (12.9)	9.9 (13.0)
Hard maple.....	1.2 (1.3)	1.2 (1.4)	1.7 (1.8)	2.1 (2.0)	2.1 (2.0)
Loess					
Honey locust.....	3.8 (3.1)	9.6 (5.8)	20.5 (11.0)	30.5 (16.8)	30.6 (16.8)
Boxelder.....	4.8 (4.6)	9.8 (7.5)	14.0 (11.2)	22.0 (18.8)	23.3 (19.0)
Sycamore.....	0.8 (0.6)	3.5 (2.8)	8.7 (9.6)	16.0 (15.8)	16.1 (15.8)
Black walnut.....	7.2 (7.8)	11.2 (14.0)	12.2 (15.9)	12.3 (16.8)	12.3 (16.9)
Buckeye.....	2.1 (1.7)	2.0 (2.2)	2.1 (2.4)	2.7 (2.1)	2.7 (2.1)
Red oak.....	3.0 (2.9)	3.9 (4.0)	5.5 (4.8)	5.3 (4.6)	5.3 (4.6)
Shellbark hickory.....	4.1 (3.2)	4.2 (4.5)	4.6 (5.3)	4.4 (4.4)	4.4 (4.4)

\* Height in partial shade given in parentheses.



TABLE V  
DEVELOPMENT OF LEAVES AND TOPS AT MIDSUMMER AND AT  
END OF GROWING SEASON

SOIL AND SPECIES	NO. OF LEAVES		AREA OF LEAVES (SQ. IN.)		DRY WEIGHT OF TOPS (GM.)	
	OPEN	SHADE	OPEN	SHADE	OPEN	SHADE
Clay						
Honey locust.....	9 (41)*	9 (31)	42.0 (70.5)	30.4 (53.4)	1.2 (3.6)	0.6 (1.7)
Boxelder.....	55 (49)	47 (78)	77.4 (264.4)	71.0 (514.5)	2.0 (11.8)	1.7 (11.5)
Sycamore.....	7 (46)	13 (33)	10.6 (233.3)	30.0 (605.1)	1.3 (6.7)	1.8 (10.3)
Black walnut.....	105 (110)	130 (124)	204.4 (448.5)	442.2 (1115.9)	8.1 (22.4)	8.2 (39.9)
Buckeye.....	14 (11)	13 (16)	6.2 (8.1)	37.0 (30.8)	0.2 (1.0)	1.7 (1.8)
Red oak.....	20 (18)	20 (31)	131.0 (202.2)	179.8 (355.6)	3.2 (8.7)	4.2 (13.1)
Shellbark hickory.	9 (6)	11 (12)	35.4 (28.3)	61.4 (34.1)	1.2 (1.4)	1.8 (1.6)
Hard maple.....	10 (15)	17 (17)	8.2 (12.2)	17.2 (47.8)	0.2 (0.6)	0.3 (2.0)
Alluvial						
Honey locust.....	14 (127)	7 (33)	36.4 (220.1)	28.6 (57.6)	1.3 (18.1)	0.6 (3.3)
Boxelder.....	30 (39)	29 (53)	16.2 (80.6)	34.2 (118.1)	0.7 (2.9)	0.4 (2.9)
Sycamore.....	7 (34)	6 (11)	22.4 (329.6)	12.4 (261.2)	0.5 (9.0)	0.3 (4.5)
Black walnut.....	121 (159)	110 (116)	380.8 (744.8)	397.8 (716.1)	10.1 (28.3)	7.4 (16.1)
Buckeye.....	14 (7)	13 (7)	26.4 (14.6)	42.8 (25.7)	1.6 (1.3)	1.9 (1.3)
Red oak.....	17 (23)	16 (18)	110.8 (229.6)	178.0 (267.6)	3.4 (9.7)	4.3 (8.1)
Hard maple.....	5 (11)	5 (13)	8.8 (23.1)	2.6 (18.6)	0.3 (0.9)	0.1 (0.6)
Loess						
Honey locust.....	26 (143)	11 (67)	45.2 (388.9)	19.1 (116.6)	2.6 (25.9)	1.0 (7.8)
Boxelder.....	74 (158)	55 (96)	243.0 (974.5)	140.0 (577.5)	5.5 (27.5)	2.6 (14.1)
Sycamore.....	13 (28)	12 (24)	121.0 (418.4)	78.0 (356.9)	2.7 (10.6)	1.4 (8.6)
Black walnut.....	113 (150)	121 (130)	467.6 (1036.1)	771.0 (1022.9)	11.2 (38.1)	14.3 (27.2)
Buckeye.....	11 (3)	13 (10)	26.0 (5.8)	39.8 (28.7)	1.5 (1.3)	1.3 (1.9)
Red oak.....	20 (15)	14 (13)	77.2 (77.8)	58.0 (52.0)	2.4 (4.3)	1.5 (2.5)
Shellbark hickory.	20 (15)	12 (9)	28.0 (31.4)	32.1 (44.0)	0.6 (2.6)	0.7 (1.4)

\* Development of leaves and tops at end of growing season given in parentheses.

summer. The retarding effects of shade are clearly shown in table IV. Maximum growth, as measured by number of leaves, total leaf area, and dry weight of tops, occurred during July and August (table V).

The roots of seedlings were consistently more extensive under full insolation (fig. 2). By midsummer their depth of penetration averaged 25, 18, and 24 inches in the clay, alluvial, and loess soils respectively, as compared with 13, 14, and 9 inches in the shade. The

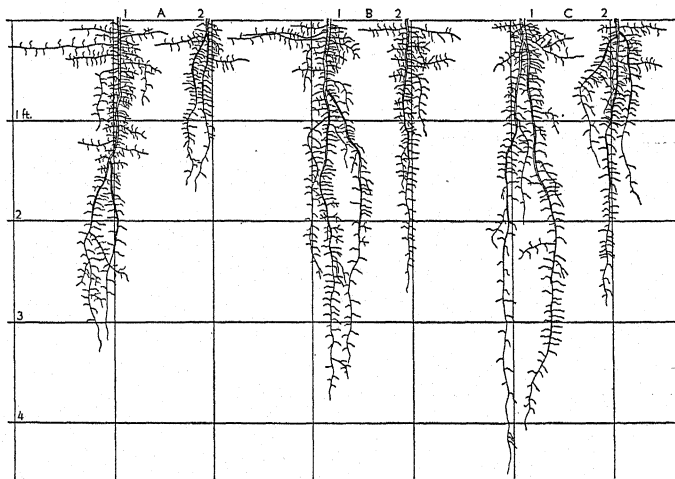


FIG. 2.—Root systems of seedling honey locusts excavated in fall from clay (A), alluvial (B), and loess (C) soils. In this and the following figures, (1) indicates trees growing in open and (2) those growing under lath shelter.

general form of these juvenile roots was the same in all cases. At the end of the season the greatest penetration was recorded in the loess and the least in the clay soil. The effects of shading were marked, especially in the clay soil where the retardation in root growth was 50 per cent.

**SAPLINGS.**—Four isolated saplings 4 to 6 years old and 4 to 7.5 feet tall were excavated, two from clay soil of upland and two from alluvial soil of low ground. The taproots had a diameter of 2 to 2.5 inches. They tapered gradually, pursued a somewhat tortuous course, and reached depths of only 20 to 24 inches in the moist soil of the lowland but 5 to 5.25 feet in the upland clay. An example of

their irregular course is illustrated by a taproot that turned at 3 feet in depth and ran obliquely upward for 25 inches, where it reached the 32-inch soil level; thereafter it pursued a vertically downward course.

Primary laterals were always abundant. From 6 to 15, exceeding 5 mm. in diameter, commonly arose from the first foot. They usually branched freely and often dichotomously. They were about twice as long in the upland as in the lowland soil, where many extended laterally 9 to 12 feet and a few over 17 feet. Usually 20 to

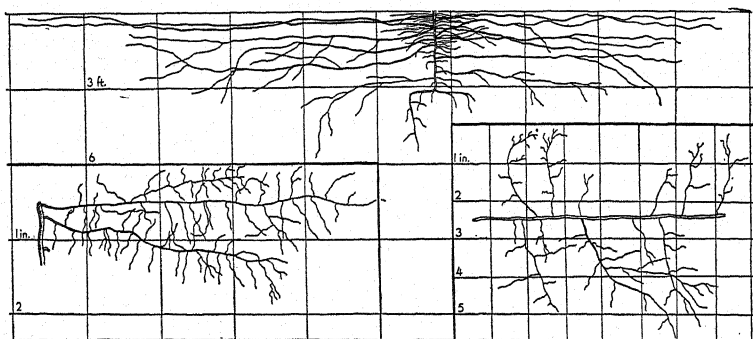


FIG. 3.—Six-year-old sapling honey locust excavated on upland (center) showing secondary laterals typical of those at 6-inch level (left), and laterals at depth of 3 feet (right).

30 smaller main laterals also arose from the first 6 inches of taproot and they occurred somewhat less abundantly to a depth of 2 feet. At greater depths they were sparse. Like the larger branches their course was mainly horizontal (fig. 3). Secondary laterals occurred at a rate of five to ten per inch of primary lateral. Tertiary branches were few and those of the fourth order rare. Even the smaller rootlets were long and of relatively large diameter. The brownish root hairs formed a thick covering on roots of the upland trees but were far less abundant on those in wet soil. The generalized, well developed root system enables this species to grow on upland as well as on lowland soil.

*Acer negundo*

SEEDLINGS.—The seedlings of boxelder made a rapid early growth in the clay and loess soils but in the wet alluvial soil growth was re-

tarded (table IV). Leaf area in the loess plots was three times greater than that in the clay plots, but on the floodplain it was much less. Dry weight of tops showed similar relations (table V). Many of the lower leaves fell from the seedlings in the clay soil during the dry period in July and August. Consequently, in early fall the leaf area in the shade was about twice as great as in the open. Leaf area

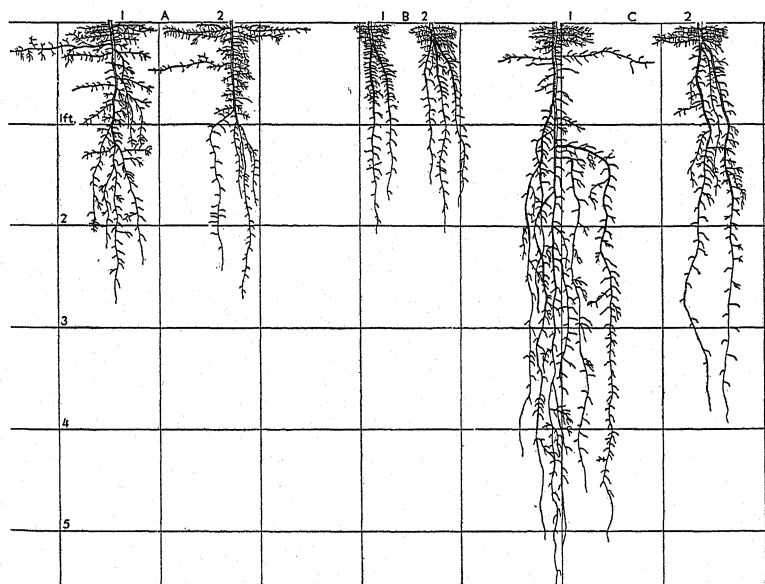


FIG. 4.—Root systems of 4-month-old boxelders grown in full sunshine and half shade in clay (A), alluvial (B), and loess (C) soils.

in the shade on the floodplain exceeded that in the open but dry weight of tops was the same under both conditions (table V). Number and area of leaves and dry weight of tops in the loess soil were almost twice as great in full insolation as in the shade.

The root systems showed similar development in midsummer, except in the wet alluvial soil where growth was much retarded. Their development by September is shown in figure 4.

**SAPLINGS.**—The root systems of two saplings about 4 inches in diameter, 13 and 15 years old and 13 and 15 feet high, were excavated near a creek where they were much shaded except on the north. Because of large lateral branches, the taproots decreased rapidly in

diameter to about 0.75 inch at 2 feet and 0.25 inch at 5 feet. The general course was not vertically downward but approximately that of a large spiral, the deviation from the perpendicular varying from 1.5 to about 3 feet. The maximum distance of the end of the taproot from a perpendicular line drawn from the soil surface was 27 inches. One taproot grew to a length of 9 feet, the other, after branching dichotomously at 7.5 feet, extended both branches to the 8.5-foot level where they ended in nearly saturated soil.

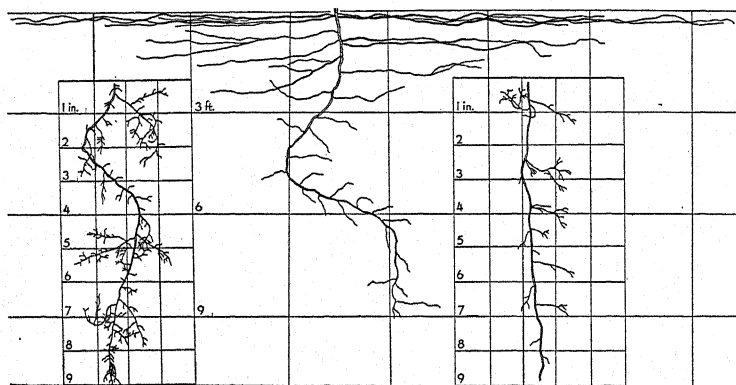


FIG. 5.—Root system of 15-year-old boxelder growing on floodplain (center) showing small portions representative of branching near soil surface (left), and at 3 feet (right). The same method of showing degree of branching of saplings is used in the following figures.

Three to six large laterals averaging about 0.75 inch in diameter arose from each taproot in the first foot of soil. They extended outward near the soil surface to a distance of 5–12 feet. Four or more other laterals, 0.75–1.5 inches thick, arose at greater depths but mostly in the surface 24 inches. They branched freely but did not spread so widely, usually only 2 to 8 feet. Their general course, like that of the shallower branches, was nearly horizontal. Frequently they divided into three or four parts of about equal size. Small primary laterals were few. Below 2 feet, all primary laterals were sparse, poorly developed, and little rebranched (fig. 5). Branches of the second and lower orders were most abundant within a few feet of the base of the tree.

The three isolated saplings examined in the clay soil of the up-

land were only 5 years old, 2 inches in average diameter, and 7 to 8 feet tall. Notwithstanding their immaturity and smaller tops,

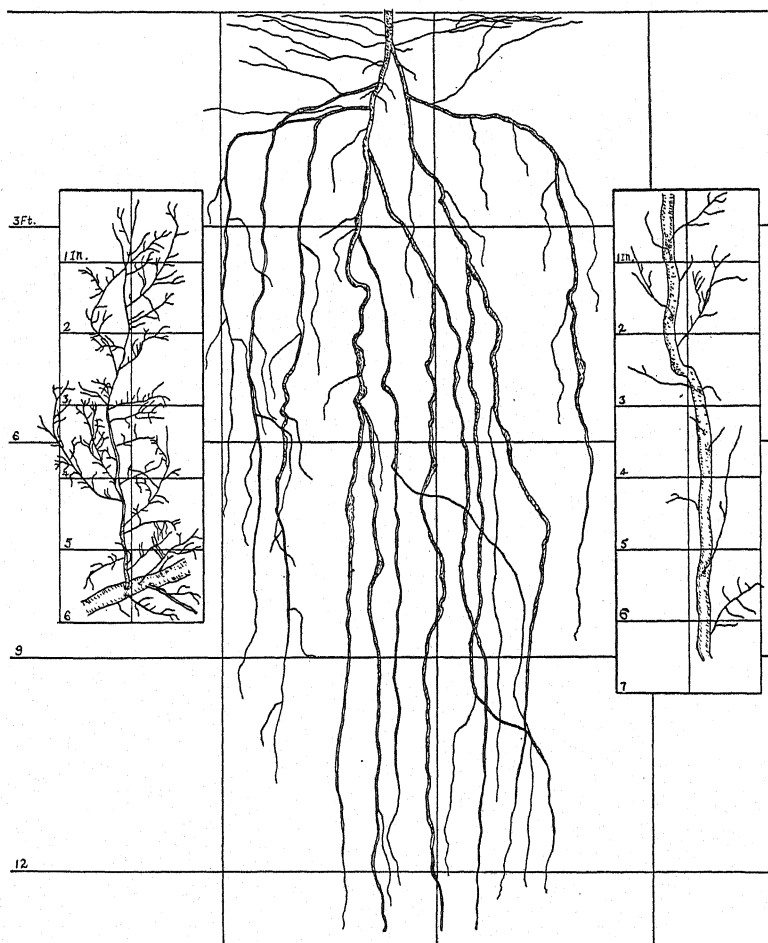


FIG. 6.—Root system of 5-year-old boxelder growing in upland clay soil. Note numerous surface roots and much distorted, deeply penetrating root system. Longest roots were traced only 12 to 13 feet but they penetrated more deeply.

their root systems greatly exceeded those of the floodplain trees. Figure 6, which is representative for the group, shows not only the greater depth of penetration, which was 12–13 feet, and the smaller

lateral spread of branches, which scarcely exceeded 4 feet, but also the vertical course and great depth to which many of the large laterals penetrated in this better aerated and drier soil. Even the smaller branches grew only a little outward and then turned downward. Close examination shows the tortuous course of the roots through the hard, rather dry clay. In cross section they often varied in shape from circular to an ellipse six times as long as wide. Below 7 feet, where the water content increased, the roots resumed a more regular shape and exhibited fewer curvatures. Finer roots were also noticeably more abundant here. On the upper portion of the taproot where the branches ran outward and upward in the surface foot, branching was especially profuse.

Grafting between roots of various sizes was found. It usually occurred where a small root crossed over or under one of larger size. Apparently the resistance between the growing roots was less than that between the roots and the soil. Where the roots of mature trees were partly exposed by soil erosion it could be seen that grafting was common.

#### *Platanus occidentalis*

SEEDLINGS.—The sycamore seedlings grew slowly during the cool early spring and developed best during August (table IV). Leaf area and dry weight of tops were greater in the shade in clay soil; otherwise they were greater under full insolation. The roots were most extensive in the loess (3 feet), intermediate in the clay (2.2 feet), and least developed in the alluvial soil (1.9 feet). Shading caused a retardation in depth and also in the production of laterals.

SAPLINGS.—The root systems of four saplings 5 to 6 years old and 7.5 to 10 feet tall were studied. The largest had a crown diameter of 8 feet. They grew in a mellow loam soil underlain at about 2 feet with a tenacious clay. The strong taproot pursued a nearly vertically downward course, tapering gradually. One taproot, for example, was 4 inches thick at the soil surface, 3 inches at a depth of 1 foot, but only 0.2 inch at a depth of 5 feet. It branched dichotomously a little deeper and ended at about 7 feet (fig. 7). The roots were often distorted in penetrating the hard clay soil.

Usually five or more large primary laterals, 0.5–0.75 inch in

diameter, arose from the first 6 inches of the taproot. These spread widely, usually 5-9 feet, and branched freely in the surface 6 inches of soil. They were supplemented by primary laterals of smaller diameters. Major roots arising in the second 6-inch soil layer or deeper usually extended obliquely downward 1-2 feet, and then turning almost vertically downward penetrated to depths of 4-6 feet. A few, however, spread irregularly or horizontally outward. One at a depth of 2.5 feet, for example, ran nearly 5 feet laterally before turn-

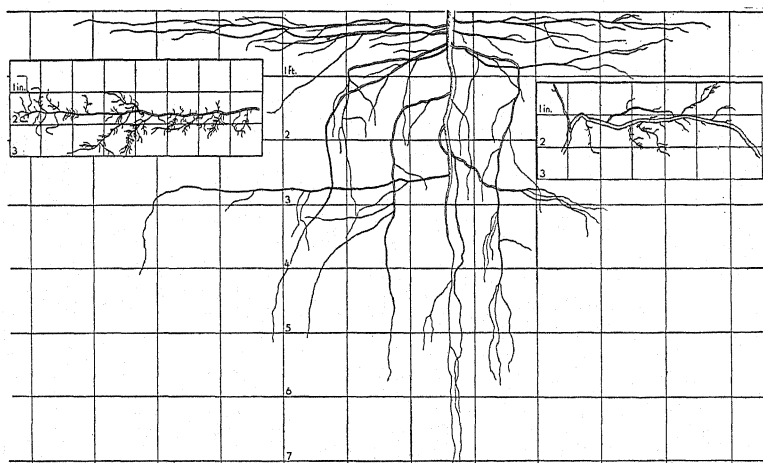


FIG. 7.—Typical root system of 6-year-old sycamore grown in loam soil

ing downward. Upon this widely extending framework, absorbing roots occurred abundantly. A maximum rate of 32 branches per inch was found in the mellow surface soil. Between the depth of 6 and 18 inches a rate of branching of about 12 laterals per inch was determined. Except for branches of the first order, laterals were scarce below 25 inches. Here many of the primary laterals were free of branches for several inches.

#### *Juglans nigra*

SEEDLINGS.—Growth in height of the seedlings of black walnut was favored in every case by shading, the effects being greatest in the clay soil. The tallest seedlings, however, grew in the loess soil. The leaf area was more than doubled, and dry weight of tops was



increased 78 per cent under shade in the clay soil. Conversely, at the other stations the number of leaves, leaf area, and dry weight of tops were greatest under full insolation (table V).

The roots were very similar in all three soils early in July, although shading caused a retardation of 3-6 inches in length, the average depth being about 27 inches. A lateral spread of 12-24 inches was attained. By the end of the summer differences in the soil had caused marked changes in the form of the root system

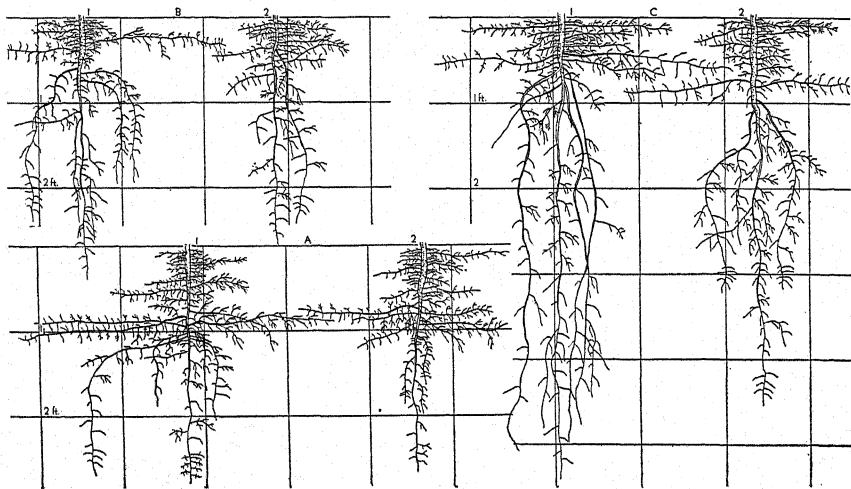


FIG. 8.—Roots of black walnut in clay (A), alluvial (B), and loess (C) soils in September of first growing season.

(fig. 8). Those in the clay spread widely, but in the loess, in addition to spreading widely, they penetrated far downward. In the alluvial soil they were somewhat intermediate in character.

**SAPLINGS.**—Three trees, 7 to 12 years old, growing in an upland clay loam underlain with a heavy clay subsoil, were selected for study. They varied from 2 to 6 inches in diameter, were 7-21 feet in height, and had a spread of tops of 5-10 feet. They were partially shaded by a second-growth forest. The plan of development of the taproot was similar in all in regard to rate of tapering, number of major laterals, and depth at which they divided into terminal branches (fig. 9). A taproot 2.25 inches in diameter decreased to 2 inches at 1 foot in depth and to 1 inch at 2 feet, where it branched into three parts, each

reaching a depth of 52 inches. The largest tree extended its taproot to 72 inches. The direct course of the roots was impeded by the hard soil, and the roots were crooked and often flattened.

The longer laterals occurred near the soil surface. On one tree, for example, six, each about 1 inch thick, arose from the first 4 inches of the taproot. Only one extended obliquely downward. It penetrated to a depth of 4 feet. Five extended directly outward along an almost horizontal course. In most cases the major laterals divided into two or three parts at a distance of 5 to 6 feet from their

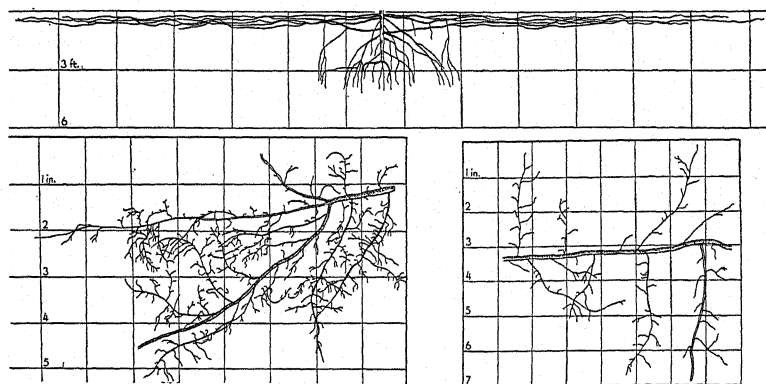


FIG. 9.—Root system of 7-year-old walnut in upland clay soil. Note wide spreading but shallow penetration of roots.

origin. These curved gracefully outward, tapered gradually, and spread widely. Some ended 16 feet from the base of the tree, one extended outward 18.8 feet, and the longest to 20 feet, which was three times the width of the entire crown of the sapling. On a tree 21 feet tall and 10 inches in diameter one of these horizontal laterals was traced outward 51 feet, where it was still 0.5 inch thick. All of these shallow roots were abundantly furnished with secondary laterals which averaged about 14 per foot but sometimes reached a maximum of 40. In many cases, however, only a few secondary branches were found on the initial 3-4 feet of main laterals.

A few vertically penetrating branches, often designated as sinkers, were found on some of these horizontal roots. They branched freely and penetrated into the third and fourth foot of soil. A few of the

deeper and smaller laterals extended upward and outward, some to within 3-4 inches of the soil surface, at 4-5 feet from the base of the tree. But most of these rather numerous laterals ran obliquely outward and downward throughout a course of 3-5 feet. None extended deeper than the taproot, or about 52 inches, and their lateral spread seldom exceeded 4 feet. Sublaterals were less abundant on these deeper branches, and were often threadlike and poorly re-branched.

*Aesculus glabra*

SEEDLINGS.—Most of the growth of the shoots of buckeye occurred in May. After the exhaustion of the food supply from the seed, development was greatly retarded. By fall they had added but little to the growth attained in June. Growth was slightly greater under full insolation in the clay and loess soils but less in the alluvial soil (table IV). Defoliation began to occur in July and by the middle of August some seedlings were completely leafless. Dry weight of tops was considerably greater in the shade in the clay soil; in the other soils few or no differences were found.

In July the roots extended downward about 19 inches in both the clay and loess soil, and 13 inches in the alluvial soil. In the shade they were not so deep. There were relatively few major laterals, the chief feature being a strong taproot. By September those in the open had penetrated to depths of 28 and 39 inches in the clay and loess soils, and to 23 inches in the alluvial soil. Little increase in lateral spread occurred throughout the summer.

*Quercus maxima*

SEEDLINGS.—Unlike the other species, red oak seedlings made their best growth in the clay and their poorest in the loess soil. Shading caused a marked increase in development of tops in both the clay and alluvial soils. Growth was very poor in the loess soil and tops were about equally developed in sun and shade (table V). Leaf area was 75 per cent greater in the shade at the clay and alluvial plots. Dry weight of tops was also greater.

Roots were most extensive in the clay soil and least in the loess at the time of the midsummer excavation. Laterals were about equally

developed in all three soils and under both conditions of light. Trees in full insolation at the clay and loess stations had a better development of laterals in the fall at which time they had reached depths of about 3.2 feet. Those in the shade were nearly as deep. Both sun and shade forms in the loess soil were less extensive, reaching only a little beyond the 2-foot level.

**SAPLINGS.**—Six saplings 5 to 8 years old and ranging in height from 3 to 12 feet were excavated. All grew on the lower slopes in clay soil and were shaded a part of each day. The strong taproots ranged

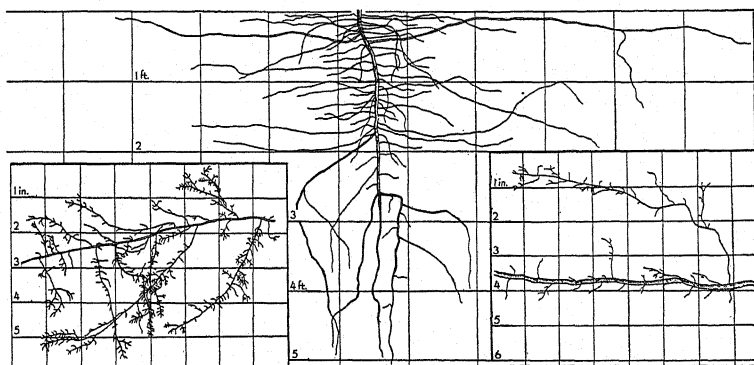


FIG. 10.—Root system of red oak in June of its fifth summer

in diameter from 0.85 to 2 inches. They tapered gradually; one 1 inch in diameter, for example, was 0.1 inch thick at 4 feet in depth and ended at 5.1 feet. A larger one extended to the 6.3-foot level. The course of the taproot in all cases was irregular, deviating 7 to 12 inches from the perpendicular. The irregular course and asymmetrical nature of the root system resulted no doubt from the hard clay soil.

The taproot was furnished with many rather horizontal branches, some of which spread widely, especially in the surface soil. Only a few of those in the surface 2 feet grew downward obliquely, but most of the roots below this level ran obliquely or nearly directly downward (fig. 10). Sixteen small branches, ranging from threadlike width to a thickness of 0.5 inch, arose from the first 6 inches of the taproot of a 5-year-old tree and 36 branches from one 7 years of age.

They varied in length from 1 to 5.7 feet. Nearly all had their entire course in the surface 6 inches of soil. Seven and 16 similar roots occurred on the second 6 inches of the two taproots, respectively, and 21 and 28 on the second foot; but branches below the 2-foot level were relatively sparse. Some of these penetrated as deeply as the taproots. Arising from this framework numerous branches thoroughly occupied the soil. The secondary laterals occurred at an average rate of 6 per inch but a maximum of 40 per inch was found. Smaller branches were also numerous, especially in the surface 2 feet of soil. Branching of roots of all sizes decreased about half below 2 feet.

*Hicoria ovata*

SEEDLINGS.—Seeds of shellbark hickory planted in the alluvial soil decayed during the wet spring. Growth in the other soils was so slow that by September the seedlings were only 4–5 inches tall (tables IV, V). The roots penetrated directly downward and their extent, as compared with that of the tops, was very great. By midsummer all the taproots had penetrated well into the second foot of soil, but in September those in the open at the loess plot were nearly 4 feet deep while those in the shade extended to 1.8 feet. In the clay soil both sun and shade forms were about 2.4 feet deep. Laterals were numerous, mostly horizontal, short, and poorly branched.

SAPLINGS.—Three trees 4–8 years old and 3–6 feet tall were excavated in upland clay soil, and one 4 years old and 4.5 feet tall in alluvial soil near a creek. All were characterized by an increase in the diameter of the taproot over that of the base of the tree in the first foot of soil, by a generally vertically downward course, a gradual tapering of the taproot, and a rather deep penetration. While the base of the tree in figure 11 was only 1.5 inches in diameter, the taproot was 1.8 inches thick in the first foot, 1 inch in the second foot, and 0.5 inch in the third foot. Except for one striking curvature it grew vertically downward, to a depth of 5.8 feet.

On all of the taproots laterals were sparse and small in the first few inches. The four to seven that did occur were relatively short and horizontal, but all were furnished with fine absorbing rootlets that extended to within 0.5 inch of the soil surface. The bulk of the

lateral roots, usually 13 to 18, arose between 6 and 17 inches' depth. Some were more than 0.5 inch in diameter. Most of the laterals arising in the first 15 inches of soil ascended somewhat and extended far outward, some to within 6 inches of the soil surface. Laterals over 5 feet long were found at this depth and others 6.3 feet long at a depth of 14 inches. All of these roots branched freely in the rich moist soil, as many as 20 branches per inch being common. They re-branched in such a manner that the absorbing area in the surface 15 inches was very extensive.

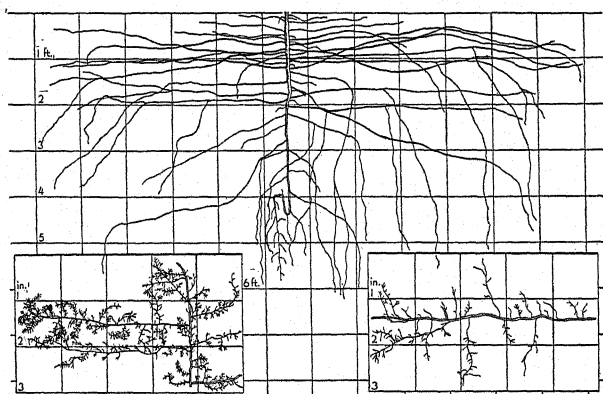


FIG. 11.—Root system of 8-year-old shellbark hickory in clay soil

With few exceptions, roots arising between the 15- and 24-inch levels extended downward. Some grew slightly downward after extending outward from the taproot 4 to 5 feet; others turned more directly downward, penetrating to an equal or even greater depth than the taproot, but rarely exceeding 6 feet. From the larger branches arising in the second foot many sinkers arose. These sometimes reached depths of 5 to 6 feet. The deepest one-third of the root system was relatively poorly branched. Thus the root system usually exceeded the top not only in length but also had about seven times as great a lateral spread.

#### *Acer saccharum*

SEEDLINGS.—The hard maple seedlings had great difficulty in forcing their large cotyledons through the soil. They grew very

slowly both above and below ground. In the clay soil they reached 2.2 inches in height in the open but were twice as tall in the shade. In the alluvial soil they were about 2 inches tall under both intensities of light. The number of leaves and total leaf area in the clay soil were about twice as great in the shade but in the alluvial soil they were almost equal (tables IV, V).

The taproots by midsummer were still within the first 6 inches of soil. In the fall those in the clay were nearly 24 inches deep but in the alluvial soil they were still within the first foot (fig. 12). In the clay, root development in the shade exceeded that in the open.

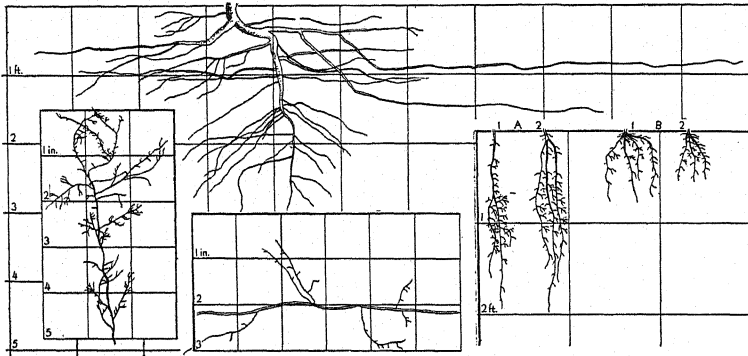


FIG. 12.—Year-old seedlings of hard maple (right) grown in clay (A) and alluvial (B) soils; root system of hard maple 16 years old (upper); and portions taken from the 6-inch level (left) and at 3 feet (lower center).

**SAPLINGS.**—Three trees, 8, 10, and 16 years old, growing on a gentle slope in clay soil were excavated. They were shaded nearly all day by a mixed deciduous forest so that height (5–11 feet) did not correlate with age. Although the taproots were 1–1.8 inches in diameter, they at once gave rise to such large laterals that their size was quickly reduced. As shown in figure 12, their course was not vertically downward. All grew obliquely downward in part, and the greatest depth of penetration was 3 feet.

Several of the shallower primary laterals on each taproot were 0.5 to 1.25 inches thick. Their general course was somewhat parallel with the soil surface, but they extended outward only 1.5–3 feet. Some of their branches, however, after descending to deeper levels

spread horizontally 5 to 7 feet. In the second and third foot branches were fairly numerous. They pursued either a horizontal or an obliquely downward course. Small rootlets were sparse, averaging near the soil surface about four per inch of main root. In the deeper soil they were even fewer and often the larger roots were bare for several inches.

*Quercus macrocarpa*

SAPLINGS.—Three 8-year-old trees of bur oak 1.5–3.5 inches in diameter and 7–12 feet tall were examined. They were growing widely spaced in upland clay soil. The strong, deeply penetrating taproots tapered gradually, in one case from 2 inches at its origin to 1.25 inches at 4 feet, to 0.25 inch at 6 feet, and to 0.12 inch at 10 feet. It tapered gradually to its end at 14.6 feet. The course was usually straight downward. Branches were numerous, frequently 18 to 24 arising from the first 14 inches of taproot. The largest were about 0.25 inch in diameter. The general direction of growth paralleled the soil surface although some roots extended upward and a few obliquely downward, some penetrating deeply. Between 14 and 24 inches numerous primary laterals 0.5–1 inch in diameter originated. These usually ran far outward, one to 11 feet. Sinkers arose from these at various intervals and penetrated directly downward to a depth of 9–12 feet. These sinkers branched freely, greatly increasing the absorbing area (fig. 13). Laterals arising at a depth greater than 2 feet extended obliquely downward, spreading only a little from the taproot and sometimes nearly equaling it in depth. The root system was well furnished with small laterals which had small tertiary branches with their sublaterals rather uniformly distributed throughout.

*Populus sargentii*

SAPLINGS.—Four saplings of cottonwood 3–7 years old and 2.5–13.5 feet high were excavated. Two were growing in a loam soil underlain with clay, and two were in clay soil. In all cases the deep taproot tapered gradually. On the largest tree, for example, it was 2.7 inches thick at the soil surface and 1 inch in diameter at 4 feet. All but one penetrated almost directly downward and reached depths varying from 5 to about 8.5 feet. One taproot divided dichotomously



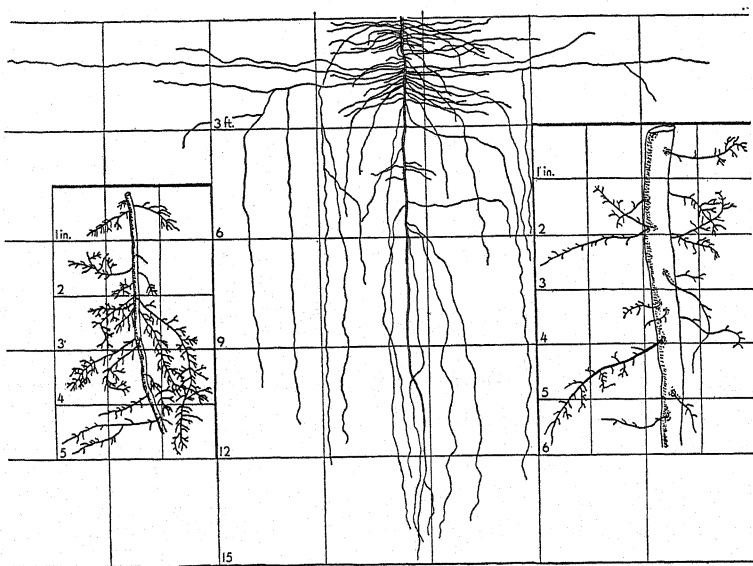


FIG. 13.—Root system of 8-year-old bur oak in upland clay soil. Note the sinkers which extend deeply and increase the absorbing system.

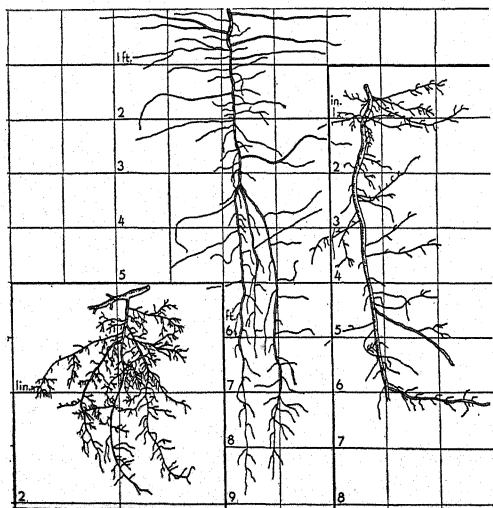


FIG. 14.—Root system of 3-year-old cottonwood

at about 3 feet, but the branches diverged only slightly from the perpendicular in their downward course (fig. 14). In the hard clay the taproots were neither smooth nor straight.

The taproots gave rise in the first 3 feet of soil to many strong laterals, some 0.5–0.75 inch in diameter, and a larger number of smaller ones. They all pursued a more or less horizontal course outward (some turning upward) to distances of a few inches to about 5 feet. Nearly all branched freely. The deeper portion of the taproot was also well supplied with primary laterals, but these were shorter and showed a strong tendency to extend obliquely downward.

Small rootlets occurred in great abundance near the soil surface, the finer ones at the rate of about 18 per inch. At greater depths these decreased in number and were more threadlike.

#### Effect of shading on transpiration

Transpiration rates of boxelder, sycamore, buckeye, red oak, and hard maple were ascertained in full insolation and half shade. The plants were grown from seeds planted in the spring in containers 5 inches in diameter and 12 inches deep. Each container had a sloping roof with a circular opening in the center 2 inches in diameter. An opening near the bottom assured proper aeration; this was sealed during the experiment. The containers were filled with surface clay soil and buried in the earth to within 1 inch of their tops. When the plants had reached a suitable size (in July), the containers were removed and sealed after thoroughly watering the plants. They were then weighed and replaced for a period of one week after which the weights were again determined. Leaf area was measured and transpiration calculated on the basis of water loss in grams per square inch per week.

Two series, consisting of six plants of each species in the open and six in the shade, were used; one from July 4 to 11 and one from July 12 to 19. High insolation was continuous during the first period. The maximum day temperatures averaged nearly 100° F. and the minimum humidities about 32 per cent. The amount of sunshine during the second period was about 90 per cent; temperatures averaged about 4° lower than for the first period, and humidity 3

per cent higher. Wind velocity was higher during the first period. Transpiration was consistently higher under full insolation (table VI).

Hard maple, one of the most tolerant species, transpired 2.5 times faster under full insolation during both periods. Red oak, also a shade enduring species, transpired 72 and 52 per cent faster, respectively, in the open during the two periods. Sycamore and boxelder, the least tolerant, lost on an average 20 and 43 per cent more

TABLE VI

AVERAGE WATER LOSS IN GRAMS PER SQUARE INCH OF LEAF AREA IN FULL INSOLATION AND IN HALF SHADE

SPECIES	JULY 4-11	JULY 12-19	AVERAGE
Boxelder.....	6.66 (5.24)*	5.03 (2.93)	5.85 (4.08)
Sycamore.....	8.04 (6.82)	7.95 (6.45)	8.00 (6.64)
Buckeye.....	7.24 (6.62)	7.25 (5.25)	7.25 (5.99)
Red oak.....	8.38 (4.87)	6.28 (4.16)	7.33 (4.52)
Hard maple.....	11.00 (4.46)	11.08 (4.43)	11.04 (4.45)

\* Water loss in half shade given in parentheses.

under full sunlight. Buckeye averaged 21 per cent more in the open. Losses during the first period were greater owing to higher temperatures, more sunshine, and stronger winds.

### Discussion

Examination of roots in midsummer showed that each species had a characteristic juvenile root habit. In agreement with the findings of TOUMEX (25), "the . . . root system. . . follows a definite course of development and maintains a characteristic form for a rather definite period of time following germination." Except for greater depth of penetration in the mellow loess soil, buckeye and shellbark hickory, which grew slowly, showed little modification under the different environments even by September. Differentiation in the honey locust was likewise largely a difference in the depth of penetration. Boxelder penetrated the loess soil twice as far as in the

clay and almost three times as far as in the alluvial soil. Sycamore showed considerable differences in root habit, branching most widely in the clay, and penetrating much farther in the loess. Differentiation in the red oak was marked, the juvenile form being retained longest in the loess soil; much branching and wide lateral spread were characteristic in the clay and alluvial soil. Walnut and hard maple showed the greatest differences in the several soil types. The largest branches of the walnut penetrated laterally far in the clay soil; in the loess they extended very deeply, somewhat parallel to the taproot. Roots of the hard maple penetrated over twice as far in the clay, where the strong branches paralleled the taproot; but in the alluvial soil the branches spread obliquely downward. Thus certain species respond more quickly than do others to changed conditions of water content, aeration, and other factors of the environment.

Species which germinate in upland soils in a relatively dry forest climate where drought in the surface layers is imminent must produce rapidly growing, deeply penetrating, and extensive initial root systems if they are to become permanently established. HOLCH (13), for example, found that the shade tolerant seedlings of *Tilia americana* could not survive in loess soil under full insolation in southeastern Nebraska, since the surface layers were depleted of their available moisture to a depth greater than that of the root system of this moisture demanding species.

The greater extent of root systems under full insolation was due largely to the fact that strong insolation promotes transpiration. The transpiration rates, while not so high as those determined by HOLCH (13) from deciduous tree seedlings in southeastern Nebraska, compared favorably with the results of WEAVER and THIEL (28), who worked on the margin of the deciduous forest in Minnesota. In the honey locust alone was growth of tops greatly retarded by shade. HOLCH, growing seedlings in different forest sites, in some cases where light intensity was very low, found light to be of major importance. WEAVER and HIMMEL (26) have shown that light affects greatly the growth of plants both above and below ground. SHIRLEY (20) demonstrated that an increase of light intensity up to 20 per cent gave a proportionate increase in growth and that root

development was always poor under low light intensity. BURNS (4) and PEARSON (18) have obtained essentially similar results.

Growth in the wet soil of the floodplain was greatly retarded during early summer. Decreased development of tops was probably partially due also to the resulting decreased absorbing area of roots. DEAN (8) found that increased aeration produced uniformly greater development of both roots and tops. HOFFMAN and SCHLUBATIS (12), working with raspberries, ascertained that the height of the water level corresponded almost as closely as did the root distribution to the type of plant developed.

Distribution of trees probably depends largely upon the adaptability of the roots to the environment. Boxelder is notably a floodplain tree. Here it usually had a rather vertically descending taproot, with large, widely spreading laterals. When found in hard upland clay, the lateral roots penetrated deeply vertically and only a little in the horizontal direction. The much flattened and distorted roots indicated that the plants were not in their usual habitat. Cottonwood roots penetrated deeply in the upland soil but also had a well developed absorbing system near the surface. The great flexibility of the root system is shown by the fact that mature trees, growing in sandy soil along the Platte River where the water table is permanently high, are sometimes blown over by the wind. They then exhibit a "flat-bottomed" root system, the laterals extending widely but the depth of penetration being shallow.

Black walnut and honey locust are not only deeply rooted but also have a widely spreading root system, and consequently a large absorbing area. This enables them to thrive in fertile upland soil as well as on the floodplain. HOLCH (13) states that 3-year-old black walnut trees penetrated beyond a depth of 9 feet and that the laterals extended to a distance of more than 7 feet on all sides of the plant. Seedlings of honey locust less than 3 months old and only 8 inches high were found by CLEMENTS *et al.* (6) to possess a taproot system 40 inches deep and widely spreading horizontal branches 6 to 18 inches long.

Hickory, red oak, and hard maple, which normally grow on moist well drained slopes in Missouri, have generalized root systems which

penetrate, in the sapling stage, about equally in all directions. Bur oak, on the other hand, occurs under the most xeric conditions and penetrates more deeply and also more widely than the preceding species. HOLCH (13) found that the first year it reached a maximum depth of 5.7 feet and a spread of almost 3 feet. In addition, the bur oak is well supplied with sinkers which add to the efficiency of the absorbing system. WEAVER and KRAMER (27) found that bur oaks 50 to 65 years old have laterals extending outward 60 feet.

The roots of the trees examined made the greater part of their growth in the vertical direction during the first two or three years. Later they developed widely spreading laterals. This is especially well illustrated by the root habits of the bur oak which are now known from the seedling stages to maturity (13, 27).

### Summary

1. Half shade favored the growth of seedlings of black walnut, buckeye, red oak, shellbark hickory, and hard maple but retarded growth of honey locust, boxelder, and sycamore.
2. Root systems were deeper and more branched in all cases where the seedlings grew in full insolation.
3. Transpiration rates were consistently higher in full insolation. Hard maple transpired 2.5 times more rapidly but boxelder only 20 per cent more than in the shade; other species were intermediate.
4. The root systems of seedling honey locusts were 1.5 times as deep as the height of the top, and those of shellbark hickory 10 times as deep; other species were intermediate.
5. Root penetration of all seedlings, except red oak, was greatest in loess soil, where depths of 36 to 65 inches were attained.
6. Total lateral spread of seedling roots varied from 6 to 18 inches, except those of black walnut which spread 4 feet in clay, 3 feet in alluvial soil, and 3.5 feet in loess.
7. Roots were most poorly developed in the alluvial soil by mid-summer, owing to deficient aeration in spring; by September honey locust and black walnut had penetrated deeper here than in the clay soil.
8. Honey locust has a generalized root system readily modified by

environment. In upland soil taproots of saplings penetrated to 5 feet, but on the floodplain they penetrated only to 2 feet, and laterals extended outward 10 to 17 feet.

9. The generalized root system of boxelder is very plastic. On the upland the taproot and nearly all the major branches penetrate deeply. In alluvial soil the root system is much shallower but extends widely in the surface foot.

10. The taproots of 6-year-old sycamore saplings penetrated upland soil to about 7 feet, branched widely at all depths, and were also furnished abundantly with deeply penetrating laterals.

11. Seven- to 12-year-old black walnut saplings developed strong taproots 4-6 feet long with many oblique branches spreading 3-6 feet and often reaching similar depths. In addition numerous strong horizontal laterals extended outward to distances two or three times the diameter of the crown.

12. The root systems of 5- to 8-year-old red oaks were deep, abundantly supplied with mostly horizontal laterals in their upper course and obliquely descending ones at greater depths. A column of soil 9 feet square and 5 feet deep was usually well occupied and a few strong branches extended 6 or more feet laterally.

13. The widely spreading root system of shellbark hickory thoroughly occupied the soil within a radius of 6 feet from the tree and to a depth of 5 feet when only 6 to 8 years old.

14. Hard maple has a relatively shallow root system, that of a 10- to 16-year-old sapling extending to depths of only 2.5 to 3 feet. Large laterals were fairly numerous; a few spread beyond 3 feet, but the smaller branches were relatively few.

15. The sturdy taproot of bur oak penetrates upland clay to 15 feet in 8 years. Laterals from the upper portion were abundant and a few spread far outward and in addition gave rise to sinkers that extended deeply. Deeper laterals usually pursued an obliquely downward course.

16. The shallow rooted cottonwood of the floodplains sends its strong taproot deeply in upland soil, at the rate of 1 foot or more each year. It is well furnished with laterals throughout but these do not spread widely for 6 or more years.

Grateful acknowledgment is made to Dr. J. E. WEAVER for outlining the problem and for direction and encouragement throughout the course of the investigation.

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## PHYSIOLOGY OF PINES INFESTED WITH BARK BEETLES

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 460

RALPH W. CAIRD

(WITH ELEVEN FIGURES)

### Introduction

The great loss of timber in the southern states due to infestation by the southern pine beetle (*Dendroctonus frontalis* Zimm.) has necessitated the search for better means of control of this insect. This physiological study was made to obtain some clue as to the factors involved in the death of the trees. The situation is complicated by the penetration of the wood by various fungi which are always associated with the beetle attack (4, 5).

These studies are a part of the program conducted by the United States Bureau of Entomology on the Bent Creek Experimental Forest near Asheville, North Carolina. The work was done in the laboratories of the Appalachian Forest Experiment Station of the U.S. Forest Service under the direction of Dr. F. C. CRAIGHEAD. The scope of the work was greatly increased by the assistance given by Dr. W. C. BRAMBLE, of the Bureau of Plant Industry, and the loan of equipment by the botany department of the University of Chicago and the Bureau of Plant Industry.

### Descriptive studies

#### METHODS FOR MOISTURE ANALYSIS

*Dendroctonus frontalis* is a primary bark beetle, capable of infesting healthy pines and maturing its brood. Severe outbreaks have been common in the Virginias and Carolinas, Tennessee, Georgia, Alabama, Mississippi, Louisiana, and Arkansas. It is especially destructive to *Pinus taeda*, *P. echinata*, and *P. virginiana* (6).

*D. frontalis* outbreaks usually center about a pine or group of pines which have been injured in some way by lightning, drought, girdling,

defoliation, fire, etc. To induce beetle attacks in the stand chosen for this study, cages of wire netting filled with bark containing beetles about to emerge were set up around the bases of scattered trees. As the beetles left the bark and attacked the inclosed portion of the tree, other beetles in the vicinity were attracted to the caged trees and the neighboring healthy ones. In this way it was possible to select uninfested trees and to keep a record of the dates of attack. Certain induced outbreaks furnished trees in the early stages of attack and others gave the later stages.

The studies reported in this paper were limited to a site of sandy clay soil about two acres in extent, which afforded a pure stand of *Pinus echinata* Mill. making rapid volume and height growth. Only dominant and co-dominant trees ranging in age from 20 to 25 years were used. These were 27 to 32 feet in height and were unbranched up to about 17 feet. Their diameter at breast height was from 4 to 6 inches and no heart wood had formed.

Many *D. frontalis* beetles simultaneously attack the tree at about mid-stem. The lower and upper portions of the trunk are usually infested a day or two later. Within three or four days after the mid-stem is infested, *Ips avulsus* Eich. is attracted to the injured tree and bores into the bark from the upper limit of the *D. frontalis* attack (20 feet) up to the leader. The two beetles cause similar injury, and in most cases are the only bark beetles involved. Usually the bark is perforated by the entrance holes from near the ground line to the leader. The holes bored through the bark are small. The size of *D. frontalis* is only about 3 mm. in length and 1 mm. in width; *I. avulsus* is somewhat smaller. The insects bore through the corky bark and tunnel in the phloem, but do not enter the wood at any time (fig. 1). The flow of resin into the wounds soon stops and the greater part of the galleries appear free from resin. At intervals along the tunnels ventilation holes to the outside are made. The entire life cycle of the beetles is completed in from 30 to 40 days, three to five broods developing in a season (6).

It was necessary to place the tree in dye solution to note which rings were carrying the transpiration stream. A method for suspending the tree in position in order to place the base in the solution was devised by Mr. HUCKENPAHLER. Two 2×4" timbers were nailed to

three trees in a line, one at 3 feet above the ground and the other at 12 feet (fig. 2). With the center tree supported in this manner, it was ready for the next step in the experiment. A section was removed from the tree by cutting the trunk through at 0.5 foot and again at 1.5 feet. After this section or disc was removed, a pail of

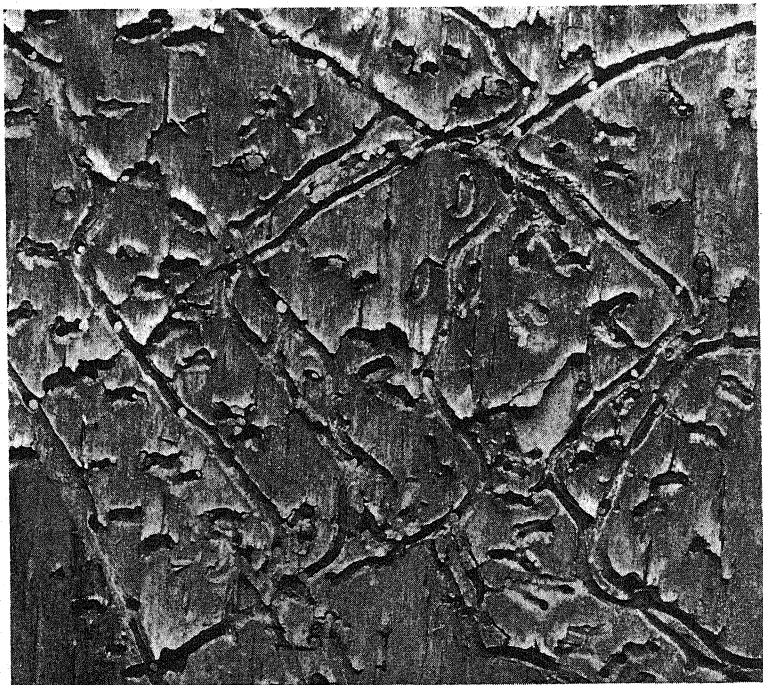


FIG. 1.—Beetle galleries and *Ceratostomella* (bluestain); tunneling in phloem by southern pine beetle and bluestain extending in vertical streaks from the galleries.

dye solution was inserted in the gap (fig. 2), adjusted so that the bottom of the suspended tree was immersed in the dye. A satisfactory dye solution contained 3 gm. of light green in 6 quarts of water.

Entrance of air into the tracheids was partially avoided by making the lower cut first and by replenishing the dye solution when necessary. The solution was often prevented from rising more than 3 or 4 feet the first day by the accumulation of resin on the cut surface. This difficulty was overcome by sawing off a disc about 1 inch

thick from the bottom of the suspended trunk each morning and evening for 3 days.

After the base of the trunk had been in the dye solution for 3 days (70 hours), the tree was taken down, limbed, and the trunk notched at 3-foot intervals in a straight line from the butt to the leader. A

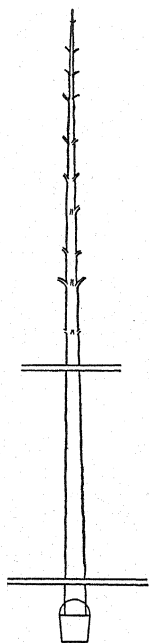


FIG. 2.—Method of suspending tree to place it in dye solution.

disc 2 inches thick for mapping and for moisture samples was removed at each notch. Diagrams showing the distribution of the dye and any discoloration due to *Ceratostomella* spp. were made by placing tracing paper over the face of the disc (fig. 3 A). The annual ring was used as the unit for sampling, rather than an arbitrarily measured depth. The tissues were grouped for moisture sampling as follows, beginning with the outside: phloem, first three rings (1-3 inclusive); second three rings (4-6); third three rings (7-9); any remaining rings in the center of the tree, called the "center" (fig. 3 B). The samples for moisture determination of the phloem were secured by removing the corky bark with a knife and stripping off the phloem. The wood for moisture determination was chipped off with a knife. The knife blade was placed next to the summerwood limiting the ring group (heavy black lines in fig. 3 B) and the wood chipped off by striking on the back of the knife. The fine chips lost water rapidly and it was found that the error involved was less if the weight of the sample was determined by weighing the disc before and after chipping rather than by weighing the chips themselves.

The samples were dried in paper bags in a large Freas electric oven equipped with a fan and regulated to  $100^{\circ}\text{C} \pm 1^{\circ}$ . Heating was continued until none of nine heavy samples from different parts of the oven lost more than 0.04 gm. after 8 hours of continuous heating between weighings. The wet weight of the samples was usually about 35 gm. The average error due to various causes was probably well within 5 per cent of the true value.

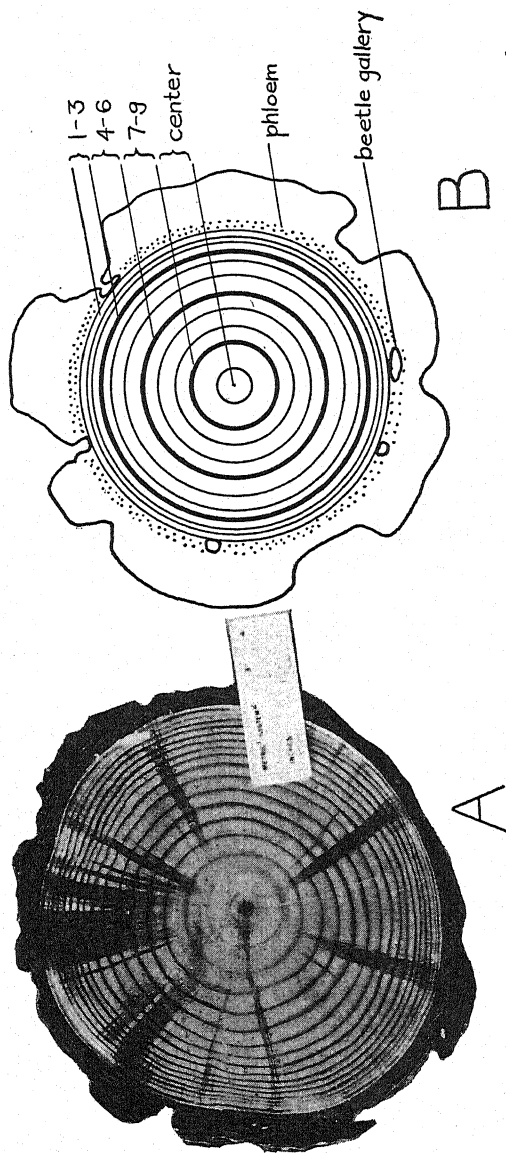


FIG. 3.—A, cross section of wood infested with *Dendroctonus frontalis* 30 days after attack (width of annual rings shown on rule); B, groups of rings taken to make composite samples for determination of moisture content.

## THE HEALTHY TREE

The conduction of dye solutions and the distribution of moisture were determined for the healthy tree in order to form a basis of comparison with the diseased tree.

DISTRIBUTION OF DYE SOLUTIONS.—The general distribution of dye in a healthy tree is shown in figure 6 A. The main course of the dye solution is up the outer rings. It passes up the inner rings more slowly, and if the tree is allowed to remain in the solution all of the rings except the center ones become colored. Vertical conduction appears to be mainly up the springwood. The dye solution which first enters reaches only 3 or 4 feet during the first 24 hours, owing to the accumulation of resin on the cut surface. During the second 24 hours, after removing a section of the base to secure a clear surface, the dye may reach mid-stem or under some conditions the branches and leaves. Irregular one-sided distribution is often observed, although more frequently the distribution is uniform. The period of three days is probably sufficient for the dye to travel as far as it will in the outer layers of the healthy and diseased trees. Distribution may be irregular during the first two days, but on the third day the outer rings are uniformly dyed. As the top of the tree is approached the dye is confined to the outer one or two rings.

It is assumed in this study, without actual proof, that the lateral movement of the dye is the same as that of the original solution. The dye may diffuse into cells which are full of sap without any movement of the water which carried the dye up the trunk. The water undoubtedly advances up the stem more rapidly than does the dye, since the wood absorbs a certain amount of the latter.

MOISTURE GRADIENTS.—The moisture content of the various layers is not the same at each height up the tree, but presents definite trends or gradients from the base to the top, as indicated in figures 4 A and 6 A. An actual tree is represented, and although it is somewhat more regular than the usual healthy tree, it shows the general relationships very well. The outer three rings have the highest moisture content of the wood samples, the values averaging from about 110 per cent (dry weight basis) at the base to 175 per cent at the top. These higher values are probably due to the immature cells

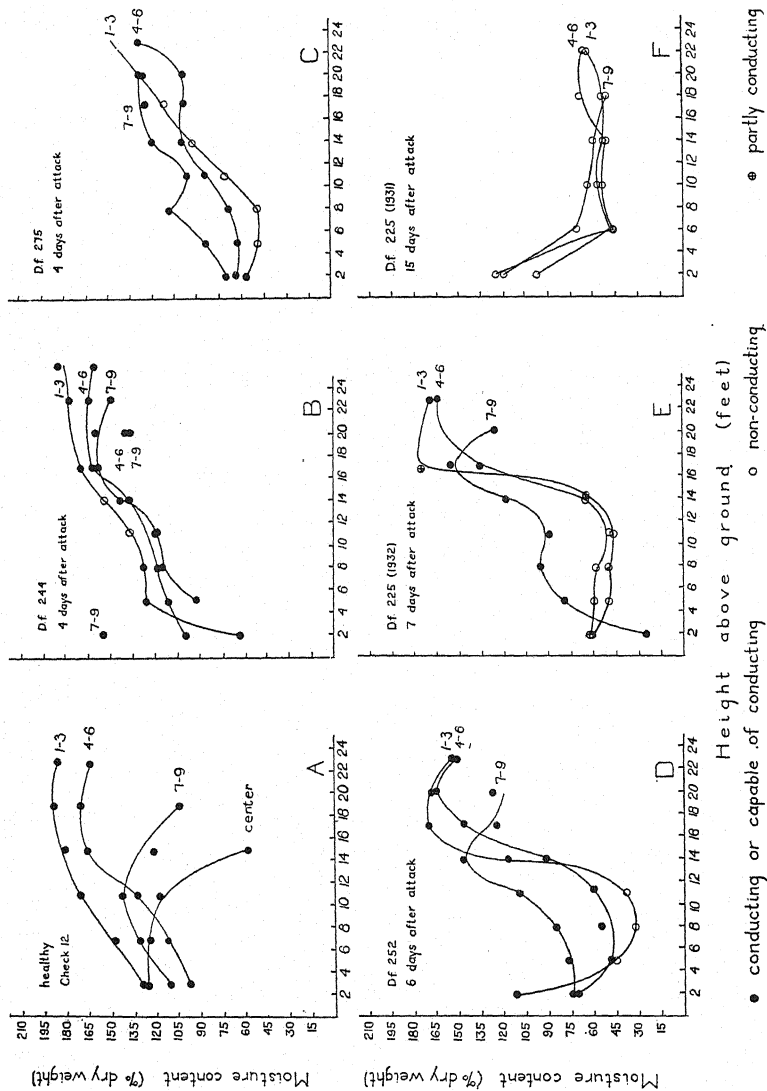


FIG. 4.—Successive stages in drying of tree and loss of ability to conduct dye solutions



adjoining the cambium and to the water of the transpiration stream. Rings 4-6 have a lower moisture percentage than rings 1-3, but have a similar type of curve. The remaining ring groups have intermediate values at the base which result in a crossing of the curves. In the top of the tree the values of all the groups are widely separated, a sharp drop in moisture content being frequently observed.

The reasons for the moisture gradients have not been investigated experimentally. The annual rings of these trees are narrow at the base of the tree and wide at the top, which probably results in denser wood at the base, leading to a lower moisture percentage. The sharp drop in the moisture content of a given group of rings when the top of the tree is approached may be the result of a functional change as these rings become the center of the tree. For example, at the base of the tree the fourth ring probably carries a portion of the transpiration stream, a function which it loses farther up the trunk. The rather slow passage of the dye solution up the center of the tree and its more rapid movement up the outer rings indicate the possibility of a gradual shift in the transpiration stream from many layers at the base to only one or two outside layers at the top of the tree.

The wide range of values obtained for the trees as indicated in figure 5 (*A, B, C*) is partly due to variations in height, diameter, and crown development, as well as to the introduction of a dye solution. Any dominant or co-dominant tree, picked at random from the stand, could be expected to have the typical graph form of the healthy tree, and to have the values fall within fairly definite limits. The diseased trees, as will be seen later, have a distinct graph form which varies significantly from that of the healthy trees. Sufficient work has been done with trees not steeped in solutions to indicate that the moisture relationships are general for the tree as it occurs in the field.

#### THE DISEASED TREE

The changes occurring when the beetles infest the healthy tree are marked. The outer rings fail to conduct dye solutions, drying takes place in these outer rings, and various fungi enter the wood.

DISTRIBUTION OF DYE SOLUTIONS.—No apparent difference from the healthy tree can be noted in the general distribution of dye in

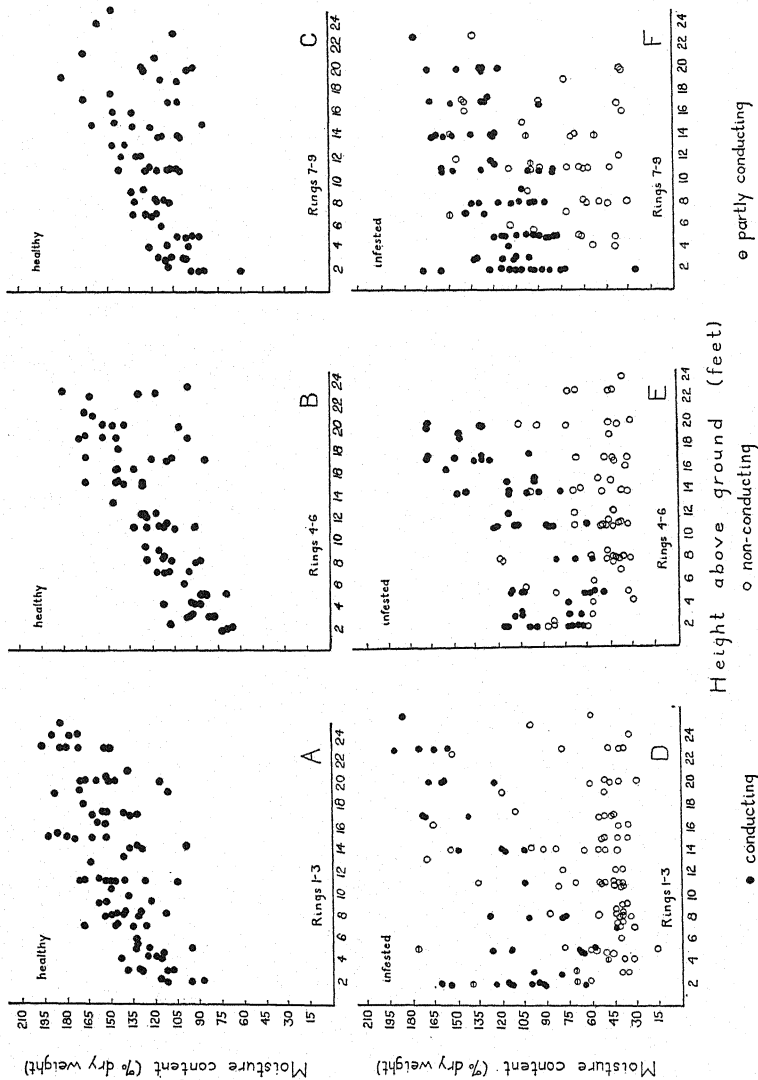


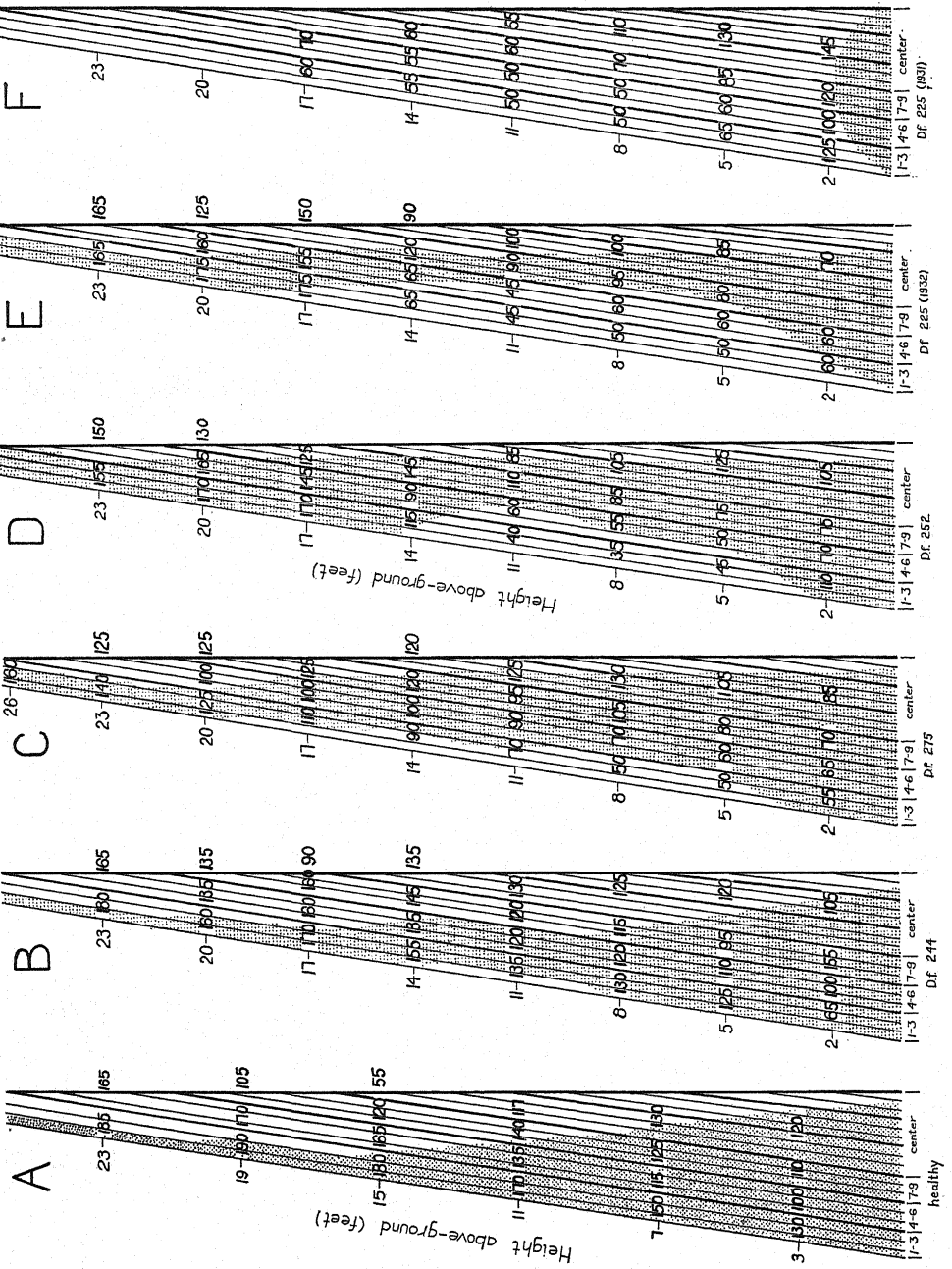
FIG. 5.—Moisture content of healthy trees as compared with trees infested with bark beetles. Percentage of moisture in wood of infested trees drops below that of healthy trees. Wood containing less than 75% moisture is incapable of conducting dye solutions.

the diseased tree during the first, second, or third days after the beetles' attack. The dye passes up to the leaves in the outer rings. Examination during the second and third days shows light colored patches in the wood along beetle galleries, caused by the accumulation of air in the cells as drying takes place. The first annual ring or two become non-conducting on about the fourth day after attack. As the outer rings become non-conducting, the transpiration stream is carried in rings which are closer and closer to the center of the tree (figs. 6, 8). In the early stages of attack the position of the dye in the top of the tree is the same as in the healthy tree. In later stages of the disease no dye reaches the top, owing to the stoppage of conduction at mid-stem.

**DRYING OF WOOD.**—The moisture content of the diseased tree soon falls below that of the healthy tree (fig. 4). The phloem becomes brown and commonly its moisture content falls from 225 to 100 per cent (dry weight basis). Rings 1-3 dry and become non-conducting before rings 4-6, and the latter before rings 7-9; that is, the drying progresses from the surface of the wood toward the center. Drying does not continue indefinitely but reaches a minimum moisture content at about 30 per cent. This lower limit is reached first by rings 1-3 at mid-stem. After 25 to 30 days the entire trunk is usually dry except for the base (figs. 4 *F*, 6 *F*). During subsequent decay the tree may become moist again, but such changes are outside this study.

Drying of the wood is correlated with loss of ability to conduct dye solutions, as is indicated in figures 4 and 5. As the wood dries it passes from a conducting to a non-conducting condition. The graphs, which contain all available data, serve to indicate a zone of demarcation lying between the wholly conducting and the wholly non-conducting values. This transition occurs in wood containing 65 to 85 per cent moisture (dry weight basis), as compared with the healthy tree values of from 100 to 185 per cent. This is a percentage figure, of course, and does not give the value of a single tracheid. The values presented are the sampling points in which the indicator dye was actually present in the wood.

**FUNGAL PENETRATION.**—The wounding of the tree by the beetles and the drying of the wood might be considered as the causes of the



stoppage of conduction, but it will be seen from the following description of the penetration of fungi into the tree that the action of the fungi might in itself account for the death of the tree. Although it seems more probable that these various factors are interdependent, each must be examined as possible single causes. It was particularly desirable to determine what fungi penetrated the wood of the diseased tree and to establish the position of the fungi with reference to the ascending dye stream.

METHODS FOR FUNGAL ANALYSIS.—In preliminary work during the 1931 season thirteen trees attacked by beetles were sampled for fungi. The trees were sampled systematically at 3-foot intervals, 50 pieces of wood tissue, or plantings, being taken from each tree with about 700 plantings in all. Approximately 1000 cultures were handled. The sampling intervals chosen were too widely spaced and the data secured were insufficient for the purposes of the investigation. The work done during 1931 was improved upon in 1932 and the results are given in detail.

Trees representing progressive stages of the disease were placed in dye solution for three days before they were taken down and limbed. The bark was scored with a hand saw in a line from the base to the leader in order to orient the discs so that they might be arranged in the same position they occupied in the standing tree. The trunk was then cut into 1-inch cross sections which were numbered consecutively according to the inches above the ground. Enough discs were taken into the laboratory to satisfy the day's requirements and the remainder were stored in a refrigerator regulated to 5° C. Tests by Dr. BRAMBLE indicate that this temperature is sufficiently low to prevent appreciable growth of *Ceratostomella pini* Münch, molds, and bacteria and yeast, if they are not kept for more than five days. The length of time needed for sampling a tree was not more, and usually less, than three days.

Wood for fungal cultures was secured by cutting a block about 1 inch wide along a radius of the disc in such a way as to include all of the rings from the bark to the pith (fig. 7 A). After transferring the block to the inoculation chamber, the bark was removed and tissue taken from the springwood of each annual ring. The wood was split by placing a sterile scalpel along the springwood of the annual ring

which was to be sampled and striking down gently with another object. Care was taken to start the splitting with a sterile scalpel and then to pry with the scalpel to complete the splitting without touching the area from which the tissue was to be taken. A second sterile scalpel was used to cut off a thin slice of wood about  $3 \times 4$  mm. in surface dimensions from the center of the exposed surface of the block. Five plantings each were taken from the first two rings, and

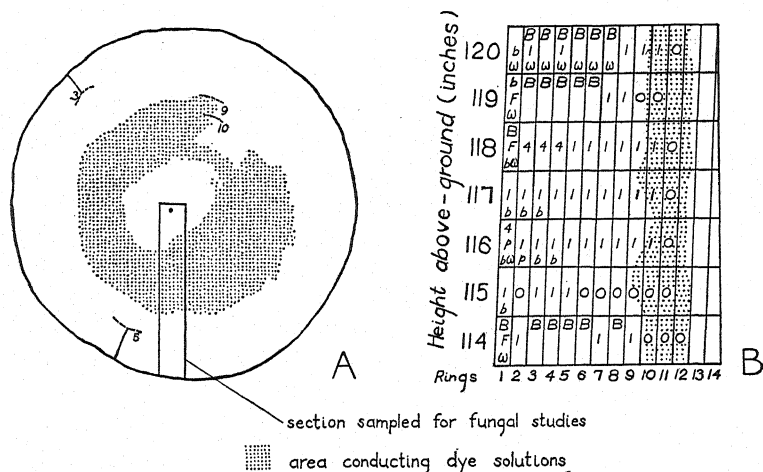


FIG. 7.—A, section of *D. frontalis* 355 at 120'' sampled for fungal studies. *Ceratostomella* apparent to the eye is indicated by heavy lines to the third and fifth rings. B, actual data collected for *D. frontalis* 355 at 114 to 120''. Key: B, *Ceratostomella* (blue-stain); r, fungus no. 1; w, bluestain wedge observable in wood; O, negative cultures; b, bacteria and yeast.

placed on malt agar in petri dishes. One planting each was taken from the successive rings toward the center until a point two or three rings within the area dyed by the indicator solution was reached (fig. 7 B). The latter plantings were placed on malt agar slants in test tubes. The tissue was pressed into the agar so as to have a portion imbedded. The only culture medium used was malt agar, upon which most fungi which decay wood will show some growth. This was prepared from Bacto-malt-agar, according to the directions supplied by the Digestive Ferments Co. of Detroit, Michigan. Asheville city water was used. The pH of the medium was not adjusted and hence was approximately 5.5. A small amount of work was done

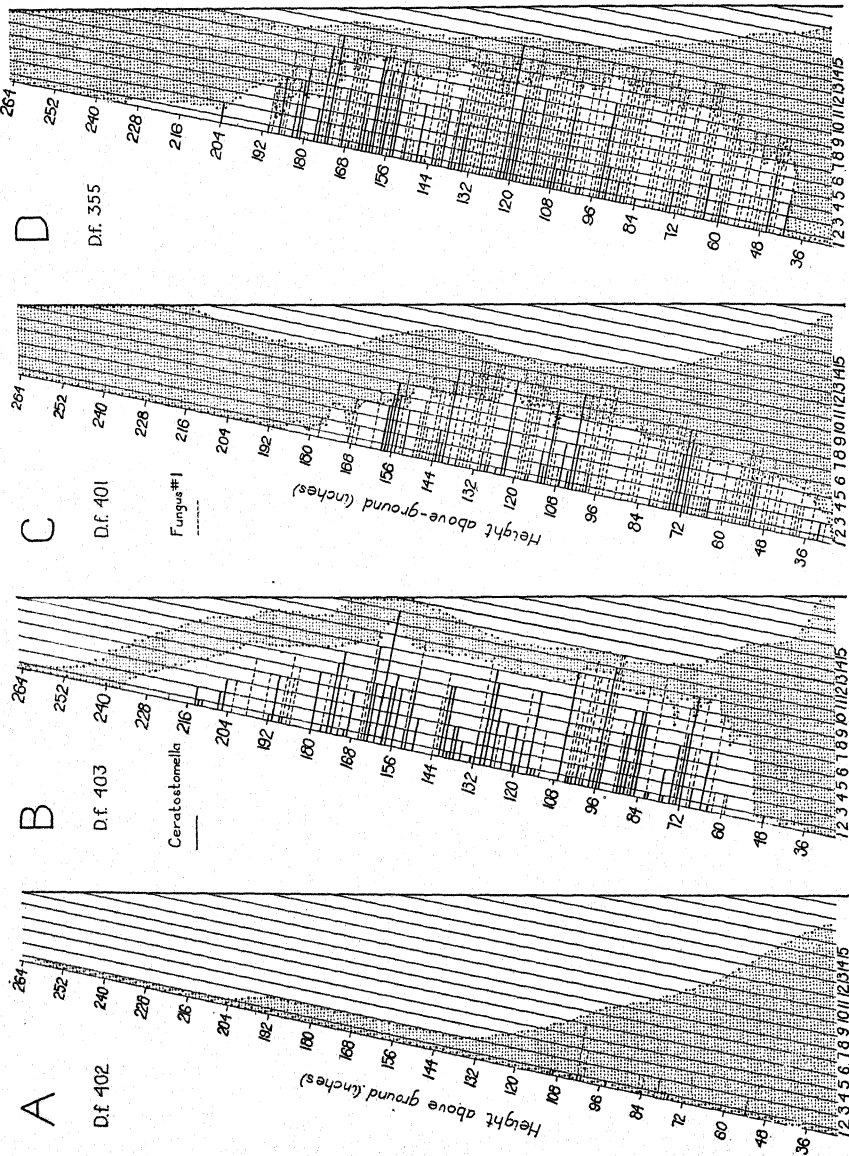


FIG. 8.—Penetration of *Ceratostomella* and fungus no. 1. Inclosed shaded area indicates wood conducting dye solutions. In *D. frontalis* 403 no samples were taken from 108 to 113", 144 to 149", or 180 to 186".

with malt agar prepared in the laboratory from a popular brand of malt syrup, using 45 gm. of syrup to 1000 cc. of water and 20 gm. of agar.

Since the data collected by these methods lose their significance if there is no assurance that the fungi isolated were actually growing in the wood and were not merely contaminations, precautions were taken to reduce the number of contaminations as much as possible. Inoculation chambers were used, the cultures were stored in large museum jars, and the mold fungi destroyed without opening the containers. The cultures were kept from 10 to 14 days, which was longer than necessary for the fungi to show distinctive colonies. Cultures designated as "sterile" at the end of this time remained so if kept for four weeks or longer. The fungi were recognized by their habits of growth on the malt agar. The great majority of the cultures appeared to be of single fungi, but no attempt was made to isolate them as such. The fungi easily recognized were *Ceratostomella* spp., *Trichoderma lignorum*, *Penicillium* sp., *Aspergillus* sp., and "bacteria and yeast." The growth habit of fungus no. 1 is distinctive although the lack of fruiting bodies has prevented its identification. No description of the growth habits of this fungus has been published.

The *Trichoderma* cultures were identified as *T. lignorum* by Dr. BRAMBLE and Dr. RUMBOLD. A culture from the isolation study designated *Ceratostomella pini* by Dr. BRAMBLE was confirmed by Dr. RUMBOLD of the Forest Products Laboratory.

The chief error in designation of the fungi was probably the failure to observe fungus no. 1 when it occurred with *Ceratostomella* (fig. 7 B). Bacteria and yeast were occasionally missed when growing with higher fungi, and it is possible that yeast with hyphae were classified with higher fungi. These errors are probably negligible when the number of cultures examined and the use to which the data are put are considered.

Figure 7 shows the detailed results upon which the description is based. Figure 7 A shows the irregular distribution of the dye and figure 7 B gives a portion of the actual notes. In making up the graphs (fig. 8) the following rules were adopted:



1. Where fungus no. 1 occurs with *Ceratostomella*, only *Ceratostomella* is shown.

2. Where fungus no. 1 or *Ceratostomella* spp. were not found in some of the rings, the fungus is shown as present in all rings to the innermost one in which it is found.

3. Where notes are lacking as to dye conduction, the points where the data are available are connected with a smooth line.

FUNGAL STUDIES IN 1932.—The results of the 1932 study are given according to individual trees which represent different stages of the disease.

Tree no. 402 (fig. 8 A) represents an early stage of attack of *D. frontalis* about three or four days after infestation. The beetles were still extending their galleries and laying eggs. These beetles which first attack the tree and make the galleries along which the eggs are laid are designated parent-adults. The tree although only lightly attacked would no doubt have died if left standing. The indicator dye appeared in the outer ring along the line chosen for analysis except at four places. *Ceratostomella* was cultured in the first ring although not apparent to the eye. Fungus no. 1 was cultured in the dyed area of the fifth ring at 102 inches and had apparently penetrated into the wood more rapidly than *Ceratostomella*. Bacteria and yeast, *Penicillium*, and *Trichoderma* were found in the first ring.

In tree no. 403 (fig. 8 B) parent-adults, tiny larvae, and eggs of *D. frontalis* were present at mid-stem and *Ips avulsus* had entered the bark from the height of 14 up to 20 feet. Close correlation between the position of the dye and the position of the fungi was not expected, and exact notes as to the position of the dye for every inch were not always noted. The dye area shows indentations associated with the presence or absence of *Ceratostomella* or fungus no. 1. This is found in 24 instances where exact notes are available. These are the only fungi present to any great extent in the inner rings of the wood. *Ceratostomella* was secured in the first ring from approximately 40 per cent of the sampling points. From 52 to 63 inches the tissue appears to be sterile for the most part but not dyed. This may be due to the heavy tunneling of the bark by the beetles and the attend-

ant drying rather than to the *Ceratostomella* and the fungus no. 1 at 64 and 65 inches. It may be that in this tree the drying of the wood has advanced faster than the fungi, and that the drying may be acting alone, apart from any action of the fungi.

Tree no. 401 (fig. 8 C) had parent-adults and eggs present at the base, one-fourth to one-half grown larvae at mid-stem, and parent-adults, eggs, and tiny larvae at the top. Plantings to the number of 2520 were taken from 25 to 185 inches. The same relation between conducting (dyed) areas and non-conducting areas appears to hold. This tree is remarkable for the small amount of *Ceratostomella* found in the inner rings. It was frequently noted as being observable on the surface of the outer rings but was actually cultured in only 37 out of 150 sampling points. Fungus no. 1 was commonly found two or three rings within the dyed area and *Ceratostomella* in only four cases. The localized effect of bluestain is again seen in this tree.

Tree no. 355 had the parent-adults which had infested the tree about 6-8 days previously. Tiny larvae were in the base and mid-stem; eggs at 15 feet and higher. *Ips avulsus* had not as yet infested the top. The fungal diagram is based upon 3120 plantings taken at 1-inch intervals along the trunk from 36 to 204 inches. As shown in figure 8 D, the dye solution was taken up in the inner rings. The outer non-conducting rings were dry and almost solidly occupied by fungi. Fungus no. 1 was cultured from the conducting and partially conducting wood in a number of instances, while *Ceratostomella* was obtained in only two cases. The small amounts encountered from 40 to 90 inches make it seem improbable that this fungus was the cause of the stoppage of conduction observed. This area was dry and fungus no. 1 was obtained from it.

The brood of tree no. 400 was in the pupal and new-adult condition, about 30 days after infestation. The tree represents a very advanced stage of the disease, in which the leaves were yellowing and conduction up the stem had completely ceased. *Ips calligraphus* pupae were in the base up to about 25 inches, with *D. frontalis* above. The tree was sampled only from 8 to 60 inches. The wood was heavily "blued" by *Ceratostomella*, and this fungus was cultured from almost every planting. *Trichoderma* was also present in almost every

planting and quickly overran the agar. Bacteria and yeast were found even in the center of the tree. Other fungi were also present, but no attempt was made to separate the fungi in the mixtures.

#### DISCUSSION AND SUMMARY OF DESCRIPTIVE STUDIES

It is obvious that an insufficient number of trees were analyzed to explore the conditions fully, although the main features are brought out.

1. *Ceratostomella* and fungus no. 1 are the only fungi which are present in considerable amounts in the interior of the diseased tree. From inoculation studies *C. pini* is known to be able to kill pines, but fungus no. 1 has not been tested in this respect.

2. The great development of *Ceratostomella* which appears as darkened wedges (fig. 3 A) does not show until late in the disease. *Ceratostomella* may penetrate the wood to the ninth or tenth rings and not be apparent to the eye.

3. *Trichoderma*, *Penicillium*, bacteria and yeast, and other fungi enter the inner wood after conduction up the trunk has been stopped. Some of the fungal forms encountered are no doubt the common fungi which rot fallen timber.

4. An unexpected, close relationship between the positions of the fungi and the outer edge of the conducting zone (dyed area) was found. A more exact study with refined technique is desirable, with careful microscopic examination of the tissue. The precise relationship between the conducting zone and the fungi cannot be drawn from this study, since the methods were not sufficiently refined and the probability of the growth of the fungi during analysis is present.

5. No moisture samples were taken of the trees examined for fungi in 1932, but the later moisture studies indicate that non-conducting zones are drier than conducting zones, and that even the outer edge of the conducting zone has lost moisture. The loss of moisture and the positions of the fungi are intimately connected; this study, of course, is merely descriptive and gives no method for establishing causal relationships. It is pointed out, however, that drying of the wood may occur without the immediate presence of any fungus, and that in this sense drying is a constant accompaniment of the stoppage of conduction but the presence (action) of fungi is not. *Cera-*

*tostomella* appears to have mainly a localized effect. It seems probable that the fungi act in some way to accelerate the rate at which the tree dries, and thus produce conditions favoring their growth.

6. The position of the fungi might be purely incidental to the stoppage of conduction, but from the inoculation studies it seems probable that the fungi are actually concerned in the death of the trees.

### Experimental evidence and general discussion

There is obviously need for experimental work to determine the action of the various factors involved when acting alone. This work is given briefly in this section, together with a discussion of the possible rôles of the various factors.

#### BEETLE GALLERIES

The entrances to the beetle galleries and the extensive tunneling of the phloem serve to expose the surface of the wood to the outer atmosphere. Drying could not occur if the bark were not opened by the beetles. This is substantiated by an experiment in which the entire bole of a pine infested with *D. frontalis* was given a heavy coating of grafting wax applied after the bark had been smoothed. New ventilation holes made by the beetles were promptly filled. The trees used were comparable intermediates about 30 feet tall and 4 inches in diameter at breast height. The moisture relationships after 33 days are shown in figure 9 A. The beetle brood failed to develop, *Ceratostomella* did not penetrate, so far as could be observed, and the leaves remained green. In a repetition of the experiment in 1932 the trunks of the waxed trees dried out, but in each case beetles made numerous ventilation holes through the wax.

The water which is lost as the trunk dries may pass out through the leaves or through the beetle gallery entrances, or both. That at least some of the moisture may leave through the entrances was indicated by a further experiment in which the trees were topped. If the limbs are removed from a beetle-attacked tree the trunk dries out fairly rapidly even though the foliage has been removed. Three pitch pines (*P. rigida* Mill.) were used in the study, the results of which are given in figure 9 B. Two of the trees had been infested by *D. frontalis* for about three days and one was a healthy tree of com-

parable size. One of the infested trees was limbed and the other was not. The limbed tree lost water from the trunk as indicated by the moisture condition after 33 days. Some water may have been lost through the cut ends of the branches but on the repetition of the experiment in 1932, trees in which the ends were covered with grafting wax showed the same general relationships as those not covered. The drying of topped trees infested with beetles has been described by NELSON in the 1929 cooperative experiment (2).

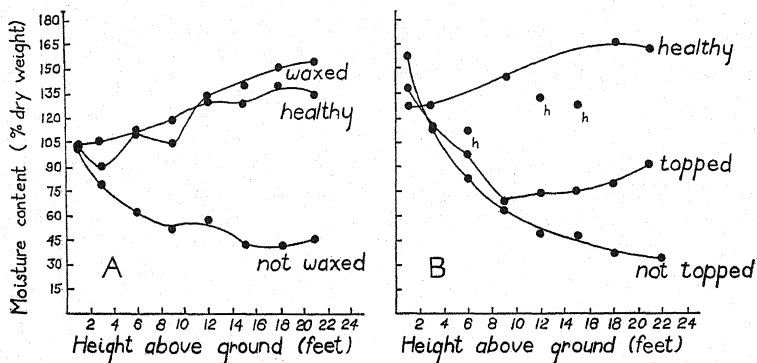


FIG. 9.—A, waxed-hole experiment: Tree infested with *D. frontalis* when waxed does not dry out to extent of a tree not waxed. B, topped tree experiment: Removal of limbs from tree infested with *D. frontalis* does not prevent drying out of trunk.

#### NATURAL DRYING

If the bark is stripped from a healthy pine for a distance of even 10 feet up the trunk, the tree does not die until long after the tree attacked by the beetles. However, the outer cells of the wood become impregnated with resin. If the surface of the wood is kept relatively dry and free of liquid resin by rubbing it with powdered resin, the outer wood dries and becomes non-conducting for the outer four or five rings, but only after a long period of time. It is possible that if the experiment were repeated and a resin-free surface maintained, more rapid drying and death of the tree due to simple drying might result and the effect of drying without the action of fungi might be determined.

## INOCULATIONS

The rôle of *Ceratostomella pini* and probably of fungus no. 1 and the other fungi appears to be the speeding up of the drying of the bole of the tree. There is a close relation between the position the fungi occupy in the tree and the drying of the wood. This physiological study was limited to the broader aspects of the disease, since the investigation of the effect of the fungi on the cells requires intensive study. From the knowledge that *C. pini* is limited largely to ray parenchyma (3), it is possible to visualize how the action of a fungus in penetrating cell walls and killing the living ray parenchyma might make easier the loss of water and the entrance of air into the tissue penetrated and into vertical elements. Throughout this study, the gases present in the cells in the dried wood are referred to as "air," without actual proof that this is true. This assumption is made from the observation of the gradual drying from the outside toward the center, and because there is no apparent suction or pressure in the dry wood on exposing dried trees to dye solutions.

When the tree is inoculated with *Ceratostomella pini* there is drying and attendant passage of the dye solution around the diseased zone which is strikingly similar to the behavior of the dye solution in the trees attacked by the beetle. This has been described by NELSON (2). HUCKENPAHLER and WYGANT inoculated four trees with pure cultures of *C. pini* and analyzed them to determine what changes had occurred with regard to dye conduction and moisture content. After 25 days the effect of a 3 inch band of inoculum (fig. 10 A) about the tree at 11 feet is as shown in the graphs of figure 11. Check trees, with comparable injury but without the fungus, were not available at the time, but later experiments showed that trees inoculated with *Trichoderma lignorum* and with sterile rice did not suffer a loss of moisture in the wood. A tree inoculated with *C. pini* and left standing had not died after one year, although it seems probable that the trees analyzed for moisture and fungi would have died. The *C. pini* was recovered from the wood by plantings but was not redetermined by an expert. Detailed moisture determinations were made of four trees in which the sharp drop in moisture content shown in figure 11 is repeated. The same type of drying occurs in trees in-

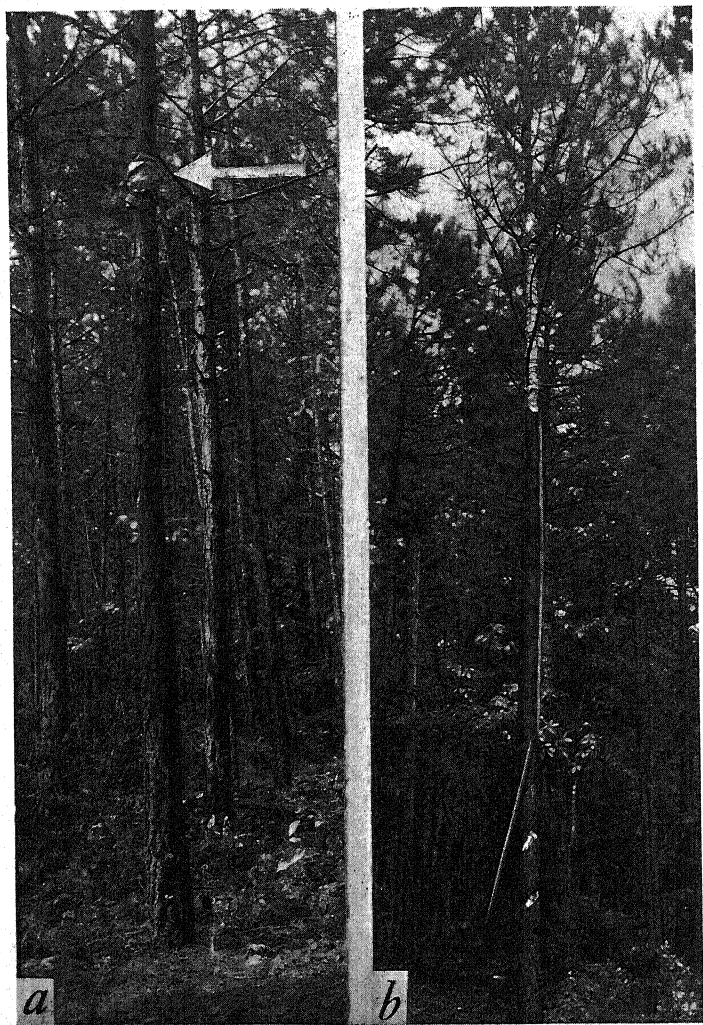


FIG. 10.—A, tree inoculated with *C. pini* in a 3 inch band at 12 feet. Inoculum applied as a poultice directly to wood by removing the bark which was then tacked back in place and the wound covered with grafting wax and waterproof cloth in an effort to prevent drying. B, tree killed by *C. pini* applied in a spiral band inoculation; tree protected from beetle attacks by wire screening and cloth.

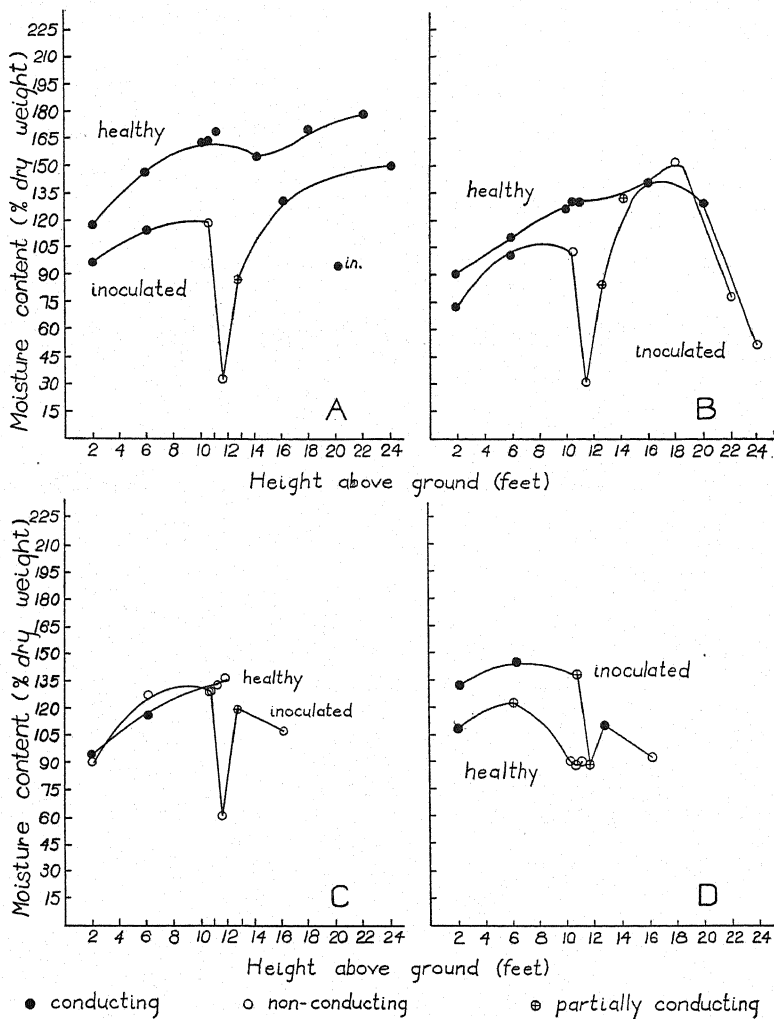


FIG. 11.—Inoculated tree no. 201 showing drying of wood at an inoculation band and loss of ability to conduct dye solutions. *C. pini* was recovered from the dried zone. A, rings 1-3; B, 4-6; C, 7-9; D, "center."



oculated with *Ceratostomella* in a spiral band (fig. 10 B), and in four bands at intervals of 2 feet. Later attempts to repeat the single band inoculations failed. The study of the tree infested with beetles, taken with the inoculation studies, serves to indicate that the death of the tree is due to the same cause in both instances. Future work might well make the inoculations a major study, since the factors can be isolated more easily.

### Summary

1. *Dendroctonus frontalis* and *Ips avulsus* bore through the bark and tunnel in the phloem. The beetle inoculates the wood with fungi and the entrance holes and beetle galleries serve to expose the wood to the air.

2. The outer rings of the tree lose the capacity to conduct dye solutions successively from the first ring toward the center. This gradually advances to the center of the tree and cuts off the supply of water to the leaves which empty the top of water and die.

3. Failure of the outer rings to conduct dye solutions is intimately associated with drying of the wood. The suggestion is made that the accumulation of air in the rings, which accompanies the loss of moisture, forms a resistance sufficient to stop the vertical and lateral transport of water.

4. The failure of the wood to conduct dye solutions is also closely associated with the penetration of the wood by fungi. *Ceratostomella pini* and fungus no. 1 are practically the only fungi which penetrate deeply into the wood during the early stages of attack. Inoculations show that *C. pini* growing alone is able to kill the trees, but fungus no. 1 has not been tested.

5. From the close connection between the drying and the position of the fungi in the wood, it is suggested that the drying is accelerated by the action of the fungi on the cells.

6. The stoppage of conduction is probably also associated with the aspiration of the tori, as indicated by NELSON's work. He suggests that blocking of the pits of the tracheids by the tori might account for the stoppage of conduction.

I have received suggestions from many with whom I have discussed the problem. Mr. R. A. ST. GEORGE and Drs. R. M. NELSON

and CARL HARTLEY have been especially helpful. The data were collected with the aid of field assistants during the summers of 1929 to 1932, inclusive, and the special contributions of these men are acknowledged in the report. Mr. N. D. WYGANT gave valuable assistance in the 1931 fungal work. The work was assisted by a Charles Lathrop Pack Forest Education Board grant, 1930 and 1932, while the writer was at the School of Forestry and Conservation of the University of Michigan.

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# MACROSPOROGENESIS AND DEVELOPMENT OF THE MACROGAMETOPHYTE OF SOLANUM TUBEROSUM

OLIVE L. REES-LEONARD

(WITH PLATES IX, X)

## Introduction

The production of fruits containing viable seeds is the exception rather than the rule in the potato. Extensive studies of microsporangogenesis and of pollen development have been made to determine the causes of non-fruitfulness, but information concerning macrosporangogenesis and the development of the embryo sac in *Solanum tuberosum* L. is limited. With the exception of the observations of YOUNG (21), apparently no detailed study of these latter phenomena in the potato has been made up to this time.

YOUNG made a brief study of the floral development, microsporangogenesis, macrosporangogenesis, macrogametophyte development, and degeneration of the sporogenous tissue in several varieties of *Solanum tuberosum*. After the archesporial cell has increased in size, he regards it as the macrospore. NANNETTI (16) concluded that the archesporium of *S. muricatum* develops directly into the embryo sac since no nuclear or cell divisions were observed that would indicate the presence of a tetrad of macrospores. BHADURI (1), in his study of *S. melongena*, traced the development of the normal 7-celled macrogametophyte which is derived from the functional chalazal spore in the linear tetrad of macrospores. KRÜGER (12) studied flower and fruit development, including macrosporangogenesis and the macrogametophyte, in several members of the genus *Solanum*. She observed that in *S. nigrum* and in *S. tubingenense* the micropylar macrospore of a linear tetrad is the functional spore from which develops a typical 7-celled macrogametophyte.

## Materials and methods

Under greenhouse conditions the buds of *Solanum tuberosum* abort early, so that it was necessary to collect material in the field. During

the flowering seasons of June, 1932 and 1933, entire flower clusters of the Irish Cobbler variety, containing buds of varied ages as well as mature flowers, were collected in the fields of Skinner's Nursery at Topeka, Kansas. In the latter part of July, 1933, material of the same variety was collected from the experimental plots of the Wisconsin Experiment Station. Flower clusters containing only small buds were placed in the fixing fluids intact. The tips of older buds were usually cut away to facilitate penetration. In cases of buds beginning to open and of mature flowers, the pistils were dissected out before being fixed.

The fixatives used were formal-acetic-alcohol (formaldehyde 5 cc., glacial acetic acid 5 cc., 70% alcohol 90 cc.), Flemming's medium solution, and Weinstein's modification of Licent's solution. The best results were obtained with formal-acetic-alcohol and with Weinstein's modification of Licent. Since the material tended to float on the surface of the solution, Carnoy's fluid containing chloroform was used for a few seconds as a "sinker," after which the material was transferred to the regular fixative to be used. After washing, the material was dehydrated, cleared in chloroform, and imbedded in paraffin.

Both longitudinal and cross sections were made at thicknesses varying from 5 to 18  $\mu$ , depending upon the size of the buds. Sections 8 to 10  $\mu$  were most satisfactory for the study of young buds, but sections of mature pistils were cut 15  $\mu$  in thickness.

### Observations and discussion

#### DEVELOPMENT OF OVULE

The ovary of *Solanum tuberosum* consists of two united carpels, each with an enlarged placenta on which many ovules are borne. The ovule initials differentiate as groups of cells from the placental tissue when the bud is a few millimeters in length. As cell divisions occur in the epidermal and subepidermal layers, the ovule initials protrude and the placenta loses its smoothness of contour. KRÜGER (12) found that in *S. nigrum* only definite cell complexes in the subepidermal layers divide to function as ovule initials, which lie distributed over the placenta.

The integument of an ovule develops as a ring of meristematic

tissue, arising near the base of the nucellus (fig. 1) at a level immediately below that of the archesporial cell. This occurs during the resting stage of the archesporial nucleus or during pre-synizesis. In its early stages the integument may be three or four cells in thickness, but when fully developed it is usually five to eight cells thick. At the chalazal end of the ovule some of the cells of the short funiculus elongate and differentiate into a vascular trace, which connects with the vascular tissue of the placenta.

The nucellar tissue begins to degenerate soon after the meiotic divisions are completed. Accompanying its degeneration, the epidermal cells on the inner side of the integument become elongated perpendicularly to form a tapetum-like nutritive layer, the cells of which possess a dense cytoplasmic content. YOUNG (21) noted that the presence of this nutritive layer in the potato was continuous in the chalazal region with a group of angular cells against which the embryo sac rests. A similar nutritive layer was observed by SVENSSON (20) in *Hyoscyamus niger*. The ovule of *S. tuberosum* takes on a curved form as a result of uneven growth of its basal portion and of the enlargement of the integuments (figs. 1-4). The mature ovule appears to resemble the amphitropous rather than the anatropous or the campylotropous type. These latter types of ovules have been reported for many of the species of the Solanaceae investigated.

#### MACROSPOROGENESIS

The single archesporial cell (fig. 2), which is usually present, is distinguished from the other cells of the nucellus by its larger size, denser protoplasmic contents, and by its greater affinity for stains. However, a multiple archesporium is frequently differentiated from hypodermal and subhypodermal cells (figs. 1, 3, 4, 7, 8). In one ovule, six archesporial cells of hypodermal and subhypodermal origin were observed. As previous observers have noted, an archesporial cell functions directly as a macrospore mother cell, not dividing to form a primary parietal cell and a primary sporogenous cell. Occasionally two, three, or four macrospore mother cells are found in early prophase stages of the heterotypic division (figs. 3, 4, 7). In the majority of the members of the Solanaceae which have been investigated, a single macrospore mother cell occurs. LESLEY (13), how-

ever, occasionally found two macrospore mother cells in an ovule of tomato. BHADURI (1) found that a multiple archesporium frequently occurs in *S. melongena*. Variations in the development of macrospore mother cells led him to conclude that "all the cells generally included under the group 'ovule initials' are potentially sporogenous."

In *S. tuberosum* there is usually but one archesporial cell which develops beyond the early heterotypic prophase. In several cases, however, two macrospore mother cells, which are in the early prophase of the heterotypic division, have been observed side by side within the same ovule (fig. 7). In another case (fig. 8) two adjacent macrospore mother cells have developed unequally; in one the homoeotypic division has been completed and cell plates formed, whereas the other has developed to the pachytene stage of the heterotypic division. In the latter cell, disintegration appears to have begun.

The functional macrospore mother cell, slightly pointed at the micropylar end, elongates until it is nearly three times as long as wide. Its granular cytoplasm becomes increasingly vacuolate but the vacuoles remain small, especially in the vicinity of the nucleus which is almost as large in diameter as the cell itself. The deeply staining nucleolus is surrounded by a light staining perinucleolar zone.

As the nucleus of the functional macrospore mother cell enters the heterotypic prophase, its resting reticulum becomes resolved into an irregular mass of leptotene threads. These threads appear to form a continuous spireme, which at first is extremely fine but later becomes thicker and contracts into a synizetic knot at one side of the nucleus (figs. 4, 5). Later the twisted and coiled threads of the spireme loosen from the knot (figs. 6, 7) and extend throughout the nuclear cavity, forming an open spireme (fig. 9). The spireme appears to be uniform in thickness throughout its length but later it shortens and thickens. Early stages in diakinesis have been observed in which several pairs of chromosomes are present, but portions of the spireme remain uncontracted. In one cell (fig. 10) nineteen pairs of chromosomes are present but several long portions of the spireme are uncontracted. The pairs of chromosomes, as seen in diakinesis, appear

short and thick, and because of their extremely small size, no constant differences in form or size could be determined.

At the equatorial plate stage (fig. 11) the axis of the spindle is parallel with the long axis of the macrospore mother cell. Occasionally a lagging of two or more chromosomes on the spindle is noted in the heterotypic anaphases.

In the macrospore mother cell shown in figure 12, the chromosomes have reached the poles and are becoming closely aggregated. Nuclear membranes inclose each daughter nucleus. At interkinesis the long spindle fibers appear to shorten and separate from the nuclei, and a cell plate is formed. By the addition of peripheral fibers, the spindle becomes barrel-shaped and the cell plate is extended entirely across the cell (fig. 13), dividing it into two daughter cells of approximately equal size. During interkinesis the two daughter cells increase somewhat in size.

Early homoeotypic prophases were not seen. The daughter chromosomes seem to move somewhat irregularly to the poles of the homoeotypic spindles. In a few instances some chromosomes precede the main group to the poles (fig. 14). In others, lagging chromosomes were observed in the late anaphases and early telophases of this division (figs. 15, 16). Lagging chromosomes were observed in both daughter cells, but they were more numerous in the chalazal cell. In several cells in both meiotic divisions it appeared that the lagging chromosomes were at some distance from the poles when nuclear membrane was beginning to form. No extra-nuclear chromosomes or small extra nuclei were observed. STEBBINS (18, 19) found that in the pistillate plants of several species of *Antennaria* irregularities in the meiotic divisions frequently occur. Lagging chromosomes are present in the heterotypic anaphases and extra nuclei are formed. FUKUDA (6) reported the presence of lagging chromosomes and extra nuclei in the microspore mother cells of potato in which the meiotic divisions occurred irregularly. He concluded that morphologically abortive pollen grains were produced by irregular and incomplete meiotic divisions.

In the Irish Cobbler the chalazal macrospore of the linear tetrad (fig. 17) always becomes the functional spore. This spore enlarges, the other three spores remaining nearly equal in size and ultimately

degenerating (figs. 18, 19). JÖNNSON (11) was the first to show that a linear tetrad of macrospores is formed in the Solanaceae. KRÜGER (12) observed a normal linear tetrad of macrospores in *S. nigrum* and in *S. tubingenense*, but, contrary to the usual condition, the micropylar cell persists as the functional macrospore.

However, variations from this regular procedure have been reported in *Solanum*. NANNETTI (16) found that the development of the female gametophyte of *S. muricatum* follows that of the so-called "tulip" type. He observed no divisions which would indicate the formation of a linear tetrad of macrospores, nor did he note any traces of macrospores which may have degenerated. Somewhat later, YOUNG (21) reported that the archesporial cell in *S. tuberosum* "grows rapidly, however, and the nucleus soon becomes quite large. At this stage it may be regarded as a megaspore; further development is delayed for a time. . . . In a few instances a row of two or three sporogenous cells was formed in the axis of the ovule, suggesting a transverse division of the original archesporial cell, though it is apparent that this is not the ordinary method of megaspore formation."

YOUNG states that, "owing to the lack of cell-division stages in the ovules in the material examined, it was not possible to determine at what stage chromosome reduction takes place, though the chromosome number was found reduced at the first nuclear division in the embryo sac." The first nuclear division in the embryo sac as shown by him (21, pl. XXVI, figs. 1, 2) resembles my figures of the heterotypic equatorial plate. The method of development of the embryo sac described by YOUNG has been interpreted by SCHNARF (17) as of the "lily?" type. The present investigation indicates that macrosporogenesis resulting in a tetrad of macrospores is the normal type of development in *Solanum tuberosum*.

These observations in *S. tuberosum* are substantiated by the condition which has been observed by KRÜGER (12) in *S. nigrum*, *S. tubingenense*, and *S. proteus*, and by BHADURI (1) in *S. melongena*. In each of these species a linear tetrad of macrospores is formed. In view of the uniform method of spore formation in all these species, it is suggested that further investigations of *S. muricatum* are necessary to determine whether NANNETTI's observations of this species



are correct or whether the normal type of macrospore formation prevails here also.

#### DEVELOPMENT OF MACROGAMETOPHYTE

The functional chalazal macrospore increases in length in part by the enlargement of vacuoles near each end of the cell. The first nuclear division occurs near the center of the cell, which has almost doubled in length. In a late anaphase of the first division of the macrospore nucleus (fig. 20), it is observed that the axis of the spindle is parallel to the long axis of the macrospore. By the time this nuclear division has been completed, the micropylar macrospores have degenerated so that only three small, lens-shaped, deeply staining bodies remain.

After the first division, the daughter nuclei, approximately equal in size, move apart and come to lie near opposite ends of the young embryo sac; between them is a large vacuolate region (fig. 21). The cytoplasm surrounding the nuclei is denser than in any other portion of the cell. At this stage the embryo sac grows rapidly both in length and in width, and becomes curved.

The chalazal and micropylar nuclei of the 2-nucleate embryo sac divide simultaneously (fig. 22). The spindles of this division are perpendicular to the long axis of the embryo sac. The resulting four nuclei (fig. 23) are respectively smaller than each of the two formed in the preceding division. The spindles persist after the formation of the four nuclei, but a cell plate has never been observed at this stage.

All four nuclei divide simultaneously, so that there are four nuclei in each end of the embryo sac. The spindles of the third division lie at either oblique or right angles to one another. In the embryo sac shown in figure 24 *B*, a portion of the spindle persisting from the second division is seen in the micropylar end of the embryo sac, extending laterally from one of the dividing daughter nuclei. One anaphase figure (fig. 24 *A*) lies so obliquely that a polar view of each end of the spindle is presented. Many young 4-nucleate embryo sacs and mature macrogametophytes were observed. The division of the four nuclei or stages showing the presence of eight nuclei preceding cell division was found, however, in only a few instances.

The four nuclei at each end of the embryo sac resulting from the

third division are connected by fibers which have persisted from both second and third division spindles. In several 8-nucleate embryo sacs, as in the one shown in figure 25, the four nuclei at the chalazal end of the sac were observed to be connected by large barrel-shaped phragmoplasts, on each of which a cell plate was in the process of formation. At the micropylar end of the embryo sac (fig. 25) there is a conspicuous phragmoplast between one pair of sister nuclei, which continues to extend laterally until it comes in contact with the wall of the cell near its tip. The spindle between the other pair of sister nuclei does not show, in this case, a cell plate. To judge from its length and position, apparently the long spindle between these two pairs of nuclei is that which persisted after the second nuclear division. An additional short spindle is seen between two adjacent nuclei.

At a later stage (fig. 26) the phragmoplasts have extended laterally to the cell wall, and a cell plate has been formed so as to cut off completely a cell at each end of the embryo sac. There is a minute groove at each end of this cell plate, apparently indicating a splitting at the margin. A few traces of spindle fibers remain. The phragmoplast with its cell plate has enlarged, curved, and extended laterally until it has reached the periphery of the embryo sac. In the micropylar end of the embryo sac, shown in figure 26 *A*, a long spindle lies parallel to the short axis of the sac between two nuclei which are not sister nuclei. The formation of the cell plate on this spindle has been delayed considerably, but it appears to extend at right angles from the vacuole to the membrane which bounds the newly formed cell just described. At the chalazal end, a membrane is formed parallel to the longitudinal axis of the embryo sac (fig. 26 *B*) between two sister nuclei and at right angles to the cell plate present on the spindle persisting from the second division. This membrane extends into the sac obliquely to meet the membrane which bounds the lateral cell already formed and thus cuts off a second antipodal cell. The cell plate on the long spindle (fig. 26 *C*) apparently will function in separating the third antipodal cell from the fourth nucleus of the chalazal group; the latter remains in the main body of the embryo sac, presumably to function as a polar nucleus.

In other views of the chalazal end of the embryo sac, the phrag-

moplasts connecting sister nuclei of the third division with their cell plates are perpendicular to each other. A cell plate is visible also on the spindle which has persisted from the second division. The three cell plates which are present delimit the three antipodals, and the fourth nucleus remains as one of the polar nuclei (fig. 27).

MOTTIER (15) found that cell division in the *Lilium* macrogametophyte results from the formation of cell plates on the spindles following the third division of the nuclei. According to McALLISTER (14), in *Medeola virginiana* evanescent cell plates are formed between each pair of nuclei resulting from the homoeotypic division, and these cell plates may persist until after the third nuclear division. JOHANSEN (10) observed that in *Hartmannia tetraptera* the fibers of the micropylar spindles concerned in the second and final division "initiate the formation of the walls of the synergids." COOPER (4) noted that in *Lilium henryi* the cells of the egg apparatus and the antipodal cells are delimited as a result of cell plate formation across the spindles of the third division and the persistent spindles of the preceding division. In *S. tuberosum* the original wall of the embryo sac, to which the cell plates extend, partially forms the boundary of each cell of the mature 7-celled macrogametophyte. The remaining boundaries of each cell apparently result from the cell plates formed on the spindles of the second and third divisions. The cell plates which are formed on the persistent spindle of the second division are delayed in their development, and appear after the cell plates are present on the spindles of the third division.

Following cell formation, the antipodal cells enlarge somewhat and conspicuous vacuoles appear (fig. 28). Indication of their early degeneration may be noted before the fusion of the polar nuclei takes place. As a result of the enlargement of the macrogametophyte, the antipodals are pushed against the chalazal tissue of the ovule and become flattened (fig. 29). The protoplasm disintegrates, stains more deeply, and the cells remain as mere vestiges or disappear entirely by the time the fusion of the polar nuclei is completed. Antipodal cells which degenerate early have been reported for *S. tuberosum* (21), *S. melongena* (1), *S. nigrum* (12), and several other genera. In *Nicotiana tabacum* (9) and *Datura metel* (7) the antipodals enlarge

and persist for some time after development of the endosperm is initiated.

The cells of the egg apparatus are somewhat elongated. In their early stages the synergids appear to be small triangular cells pointed at the micropylar end. A nucleus lies in the dense cytoplasm near the center of each (fig. 28). By continued growth the synergids elongate, the chalazal ends become slightly broader and rounded, and a large vacuole forms in the basal portion of each cell. A nucleus remains in the dense cytoplasm immediately anterior to the large vacuole. The micropylar ends of the synergids elongate, become pointed, and extend a short distance into the micropyle. The synergids develop a distinct filiform apparatus whose striations arise in the vicinity of the dense cytoplasm anterior to the nucleus and extend to the apex of each cell (fig. 29). These striations appear as dense areas in the cytoplasm, alternating with elongated vacuoles, both of which extend parallel to the long axis of the cell. A similar condition appears in *Lycopersicon esculentum* (3).

Simultaneously with growth of the synergids, the egg increases in size until its broad basal portion extends into the gametophyte beyond the synergids (figs. 28, 29). A large vacuole develops in the apical end of the mature egg, whose nucleus remains imbedded in the denser cytoplasm of the broad basal region. The egg nucleus is somewhat larger than that of a synergid.

In the mature macrogametophyte, one polar nucleus is derived from the group of four nuclei present at each end of the embryo sac. The chalazal polar nucleus migrates to a position just posterior or lateral to the egg where it meets the micropylar polar nucleus. In some embryo sacs both polar nuclei move toward each other, meeting near the center of the sac. They remain closely appressed for a short time before fusing. Fusion of the polar nuclei occurs before fertilization. The large fusion nucleus usually occupies a position just below the egg, so that it is partially obscured in sectional view by this cell (figs. 29, 30). Fusion of the polar nuclei has been found to be completed before fertilization in *Datura laevis* (9), *D. metel* (7), *Lycopersicon esculentum* (3), and *Solanum proteus* (12). Not only this fusion but also the first division of the primary endosperm cell as well occurs before fertilization in *Petunia*, according to FERGUSON (5).

## DEGENERATION

Macroscopic evidences of degeneration in *Solanum tuberosum* may be observed in buds of all sizes as well as in open blossoms. The buds which degenerate cease to grow, become yellowed, and separate from the plant at a definite abscission region in the pedicel. If anthesis occurs, many of the flowers wither very soon. In some flowers which open, the anthers appear to be shriveled and twisted. Fruits were never observed on the plants from which the material was collected.

In many buds the microsporogenous tissue has broken down completely, leaving only a densely staining mass of material in the locules of the anthers. In some of the ovules of these buds, the macrospore mother cell appears to be arrested in its development, as indicated by its unelongated condition and its deeply staining protoplasm. In some older buds the anthers appear normal in shape and size. Even in these, however, many of the microspores are shrunken and disintegrated. In these buds many of the ovules contain embryo sacs which appear to be normal; evidently not all the ovules of an individual pistil may be involved in disintegration. Pollen tubes were never observed in the styles of pistils which had been dissected out of open flowers. YOUNG (21) found that the changes which indicate degeneration in the ovules usually accompany those irregular meiotic divisions which occur frequently in the pollen mother cells; disintegration within the ovules is less frequent than that which occurs within the anther.

Degeneration may occur within the ovule of *S. tuberosum* at any stage, but it is rarely observed in very young stages. When a multiple archesporium is present, with occasional exceptions all but one of the archesporial cells disintegrate or fail to continue their development, a single cell then remaining to function as a macrospore mother cell. In some ovules meiotic divisions occur, but all four macrospores disintegrate instead of the usual three micropylar spores. In a few ovules the micropylar daughter cell was observed to degenerate completely after the heterotypic division, but the nucleus of the chalazal daughter cell completed the homoeotypic division. Cell division followed and two macrospores were formed.

Disintegration occurring immediately after the meiotic divisions, and resulting in complete disorganization of the macrospores, may be the result of irregularities in chromosome distribution. Lagging chromosomes on both heterotypic and homoeotypic spindles were observed in some ovules (figs. 15, 16). Whether or not such chromosomes are included in the daughter nuclei or remain in the cytoplasm to form supernumerary nuclei could not be determined, since many of the cells degenerate to such an extent that all internal structure is obliterated.

When degeneration takes place in the young macrogametophyte, the embryo sac shrinks away from the inclosing integument, stains heavily, and presents a shriveled disorganized appearance. The shrinkage begins usually at the micropylar end of the sac. Deep-staining masses are present within some ovules which may be 2- or 4-nucleate embryo sacs that have been almost completely disorganized. In other instances the micropylar nuclei of the young macrogametophyte were observed to have disintegrated completely, while those at the chalazal end appeared nearly normal. The cytoplasm in such cases becomes fibrillar in appearance and stains more heavily than in the normal macrogametophyte. This suggests that degeneration may not be a sudden occurrence involving the entire gametophyte at the same time, but rather may be progressive (fig. 31). In some cases the integumentary tissue inclosing a degenerating embryo sac is normal in appearance; in other ovules the nutritive cells of the integument degenerate completely with the embryo sac they inclose.

Degeneration may follow the third nuclear division in the embryo sac (fig. 32). The peculiar shape of the embryo sac here shown is due to its shrinkage within the ovule as well as to the plane in which the section was cut. A dense cytoplasmic zone in which are distinguishable many small granules is present in the center of the sac and is surrounded by large vacuoles. Eight nucleoli and a delicately staining zone inclosing each are the only visible traces which remain of the nuclei of the macrogametophyte. Apparently degeneration had set in before the initiation of cell division. BRADBURY figured a similar degree of degeneration in the egg nucleus and the fusion nucleus in

unpollinated blossoms of *Prunus cerasus* (2, pl. 53, fig. 17). The nucleoli are the only distinguishable remains of the egg nucleus and the fusion nucleus.

In some embryo sacs the micropylar ends of the cells of the egg apparatus stain very heavily and all structural details are obliterated. Only the nucleoli can be differentiated within the nuclei. Occasionally nuclear material is visible about the nucleolus of the egg, although scarcely distinguishable from the surrounding cytoplasm.

In one mature megagametophyte, which appears to be degenerating, the egg occupies a rather unusual position (fig. 30). It appears to lie at an angle of about  $160^\circ$  from the normal. The apex of the cell is directed toward the side of the primary endosperm cell, and the broad curved end lies above the synergids. The nucleus appears densely granular and has a greater affinity than usual for stains. The primary endosperm nucleus may be seen below the egg. The synergids lack a distinct filiform apparatus, but the cytoplasm at their micropylar ends is somewhat reticulate and stains heavily. The reticulate nature of the cytoplasm of the synergids, the altered position of the egg, the granular appearance and deeply staining quality of the egg nucleus, and the increased vacuolation of the embryo sac may be considered evidences of degeneration. The breaking down of the cells, however, may be a result of the failure of fertilization.

These irregularities which occur during microsporogenesis and the development and maturation of the megagametophyte may partially account for the failure of seed and fruit development in the potato. There seems to be some correlation, however, between the irregularities occurring during microsporogenesis and the development of the pollen grains on the one hand, and those taking place during megasporogenesis and megagametophyte development in the ovules of the same flower on the other hand. Further investigation should determine if possible the relationships of these irregularities to the sterility which is so common in the potato.

### Summary

1. A single archesporial cell, hypodermal in origin, may function as a megaspore mother cell. Frequently, however, a multiple arche-

sporium is differentiated from hypodermal and subhypodermal cells of the nucellus. In such case each archesporial cell may function as a macrospore mother cell, but usually only one of them so functions.

2. A linear row of four macrospores is formed. The chalazal macrospore develops into an embryo sac; the other three spores disintegrate.

3. The micropylar cells of the nucellus break down and the developing embryo sac comes into contact with the inner epidermis of the integument, which functions as a nutritive layer.

4. In the 4-nucleate embryo sac the spindle persists between each pair of sister nuclei, but no cell plate has been observed at this stage.

5. A typical 8-nucleate embryo sac is formed, at each end of which the four nuclei are connected with one another by fibers from the spindle of the third division as well as by persistent spindle fibers from the second division.

6. Cell division occurs by the formation of cell plates across the spindles of the second and third divisions in the embryo sac.

7. Changes involving degeneration within the ovule may occur at any time during macrosporogenesis and during development of the macrogametophyte.

8. Macrosporogenesis may be accompanied by irregularities in chromosome distribution. Chromosomes, lagging on the spindle, were observed in several instances in both heterotypic and homoeotypic divisions.

9. Degeneration in the microsporogenous tissue may be associated with corresponding changes in the ovules. If degeneration occurs late in the anthers, only a small proportion of the developing embryo sacs give any indication of breaking down.

10. Degeneration of mature macrogametophytes occurs frequently.

11. Pollen tubes were not observed in the style of any pistil examined.

12. Degeneration within the ovules may account to a certain degree for the failure of seed formation in the potato.

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#### EXPLANATION OF PLATES IX, X

All drawings were made with an Abbé camera lucida at table level. Bausch and Lomb compensating oculars, a Leitz periplan 20× ocular, a Bausch and Lomb 4 mm. objective, and an F. Koristka oil immersion 1/12", 1 mm. omog. AP. N. 1.36 objective were used, except for figures 26 and 27, which were made with Spencer compensating oculars and a Leitz oil-immersion N.A. 1.32 objective.

#### PLATE IX

- FIG. 1.—Very young ovule containing two archesporial cells. ×425.
- FIG. 2.—Young ovule containing a single archesporial cell. ×425.
- FIG. 3.—Young ovule containing four archesporial cells. ×425.
- FIG. 4.—Young ovule containing two macrospore mother cells, nucleus of each being in an early meiotic prophase. ×425.
- FIG. 5.—Synizesis. ×1050.
- FIG. 6.—Nucleus recovering from synizesis. ×1050.
- FIG. 7.—Two adjacent macrospore mother cells with nuclei recovering from synizesis. ×1050.
- FIG. 8.—Macrospore mother cell with nucleus in open spireme adjacent to a linear dyad in a late telophase of homoeotypic division. ×800.
- FIG. 9.—Open spireme. ×1750.
- FIG. 10.—Early diakinesis with 19 pairs of chromosomes and portions of spiremes present. ×1750.
- FIG. 11.—Macrospore mother cell with heterotypic equatorial plate. ×1050.
- FIG. 12.—Macrospore mother cell within ovule; heterotypic telophase showing cell plate formation. ×1050.
- FIG. 13.—Interkinesis; spindle showing cell plate formation. ×425.
- FIG. 14.—Daughter cells during homoeotypic division; early anaphase. ×1050.

FIG. 15.—Daughter cells during homoeotypic division; anaphase stage with chromosome lagging on spindle of each cell; cell plate forming in chalazal cell.  $\times 1050$ .

FIG. 16.—Daughter cells during homoeotypic division; early telophase stage with chromosomes lagging on spindle of each cell.  $\times 1050$ .

## PLATE X

FIG. 17.—Daughter cells formed after homoeotypic division is completed; cell plates across each daughter cell.  $\times 1050$ .

FIG. 18.—Typical linear tetrad of macrospores with three micropylar spores beginning to disintegrate.  $\times 1050$ .

FIG. 19.—Typical linear tetrad of macrospores, chalazal spore enlarging and three micropylar spores disintegrating.  $\times 1050$ .

FIG. 20.—Division of macrospore nucleus.  $\times 1050$ .

FIG. 21.—Two-nucleate embryo sac; nuclei in early prophase stage of division.  $\times 800$ .

FIG. 22.—Embryo sac; division figures present, leading to 4-nucleate stage.  $\times 1050$ .

FIG. 23.—Four-nucleate embryo sac; persistent spindle between sister nuclei.  $\times 800$ .

FIG. 24.—Division stages leading to 8-nucleate embryo sac: *a*, polar view of late anaphase of dividing nucleus; *b*, portion of spindle persistent from second division. Composite drawing from two serial sections of same embryo sac.  $\times 800$ .

FIG. 25.—Division stages leading to 8-nucleate embryo sac; phragmoplasts between sister nuclei at chalazal end and one pair of sister nuclei at micropylar end of embryo sac; spindles present between remaining nuclei in micropylar region. Composite drawing from two serial sections of embryo sac.  $\times 800$ .

FIG. 26.—Four nuclei in each end of embryo sac; antipodals and egg apparatus in process of formation by means of cell plates present on spindles of third division and those persisting from second division. Composite drawing from two serial sections of embryo sac.  $\times 725$ .

FIG. 27.—Chalazal end of embryo sac: *a*, cell plate on spindle between sister nuclei, polar nucleus and an antipodal nucleus; *b*, cell plate on spindle persisting from previous division.  $\times 725$ .

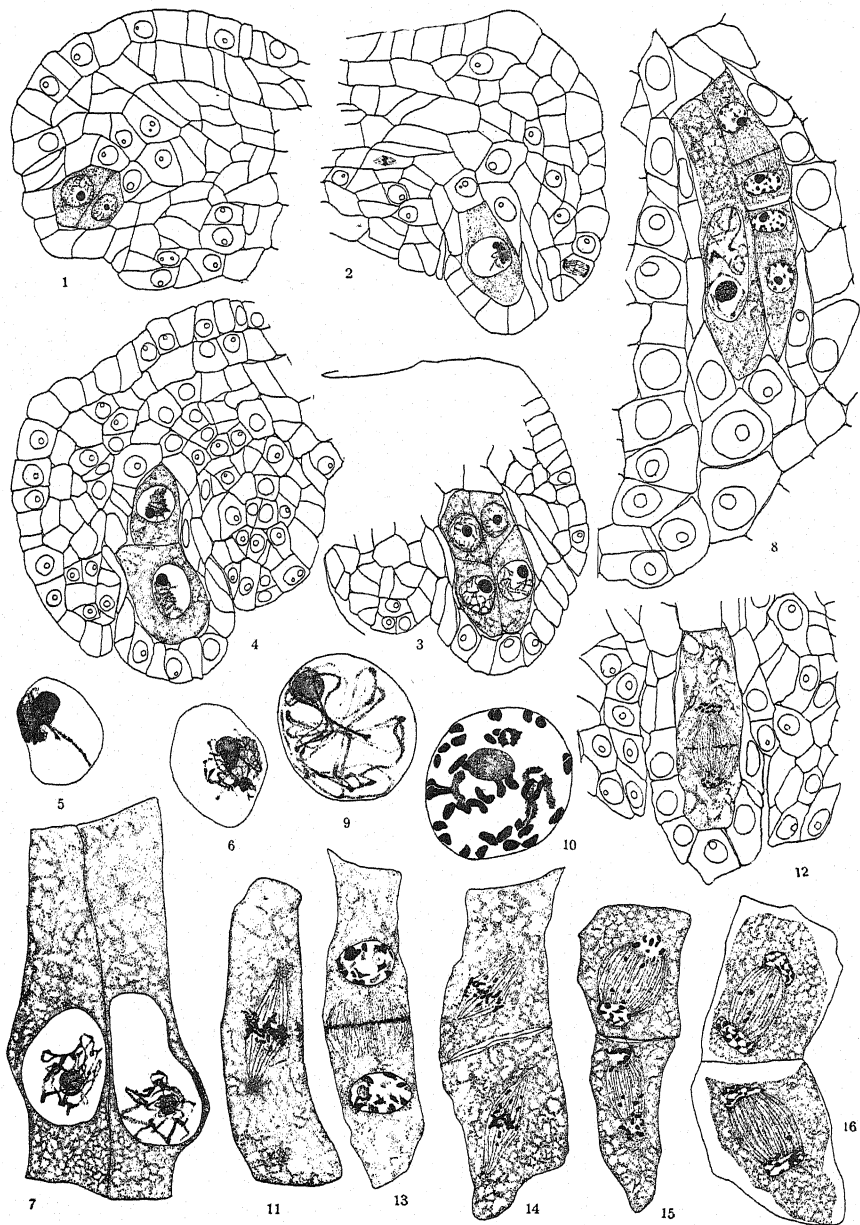
FIG. 28.—Immature 7-celled macrogametophyte. Composite drawing from two serial sections of embryo sac.  $\times 800$ .

FIG. 29.—Mature macrogametophyte, antipodals disintegrating.  $\times 800$ .

FIG. 30.—Apical portion of embryo sac showing micropylar apparatus with fusion nucleus; position of egg altered.  $\times 800$ .

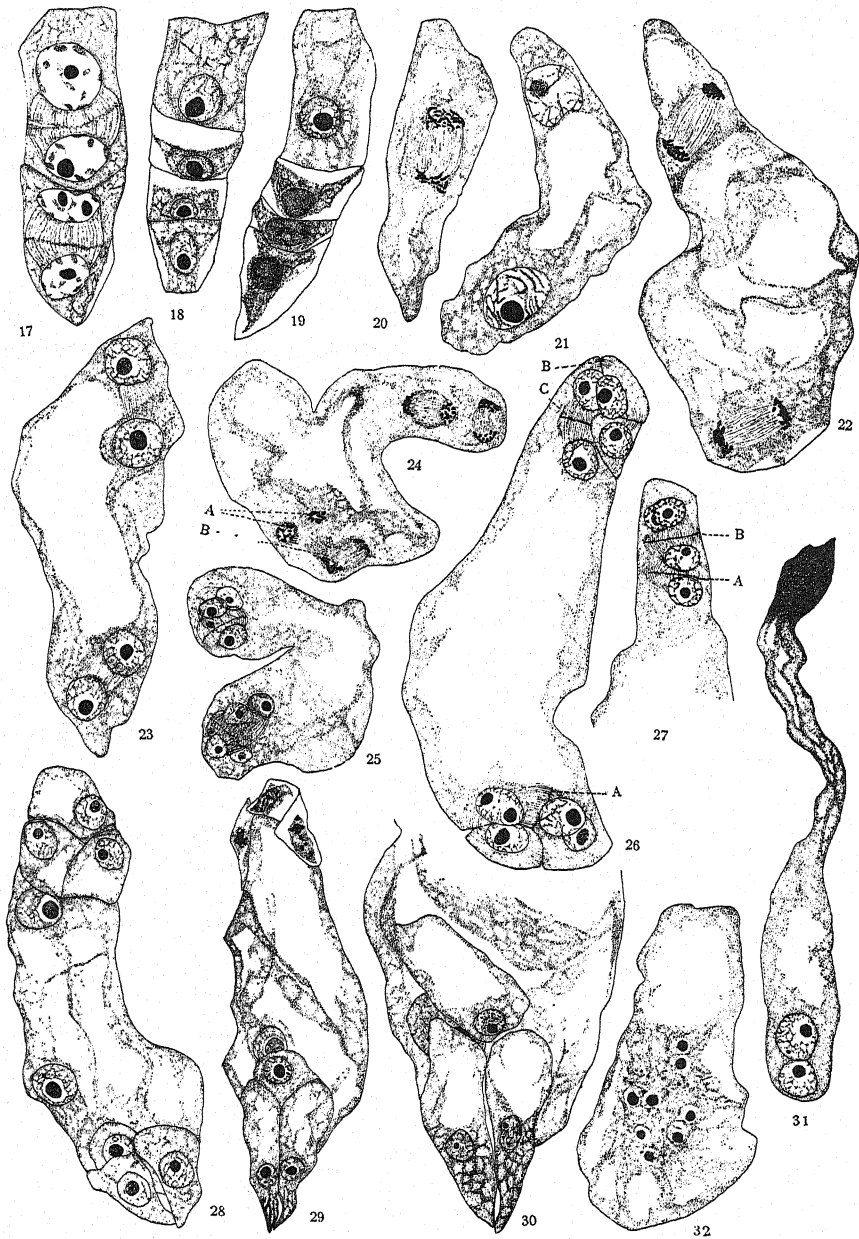
FIG. 31.—Disintegration of 4-nucleate embryo sac; micropylar end of sac involved.  $\times 800$ .

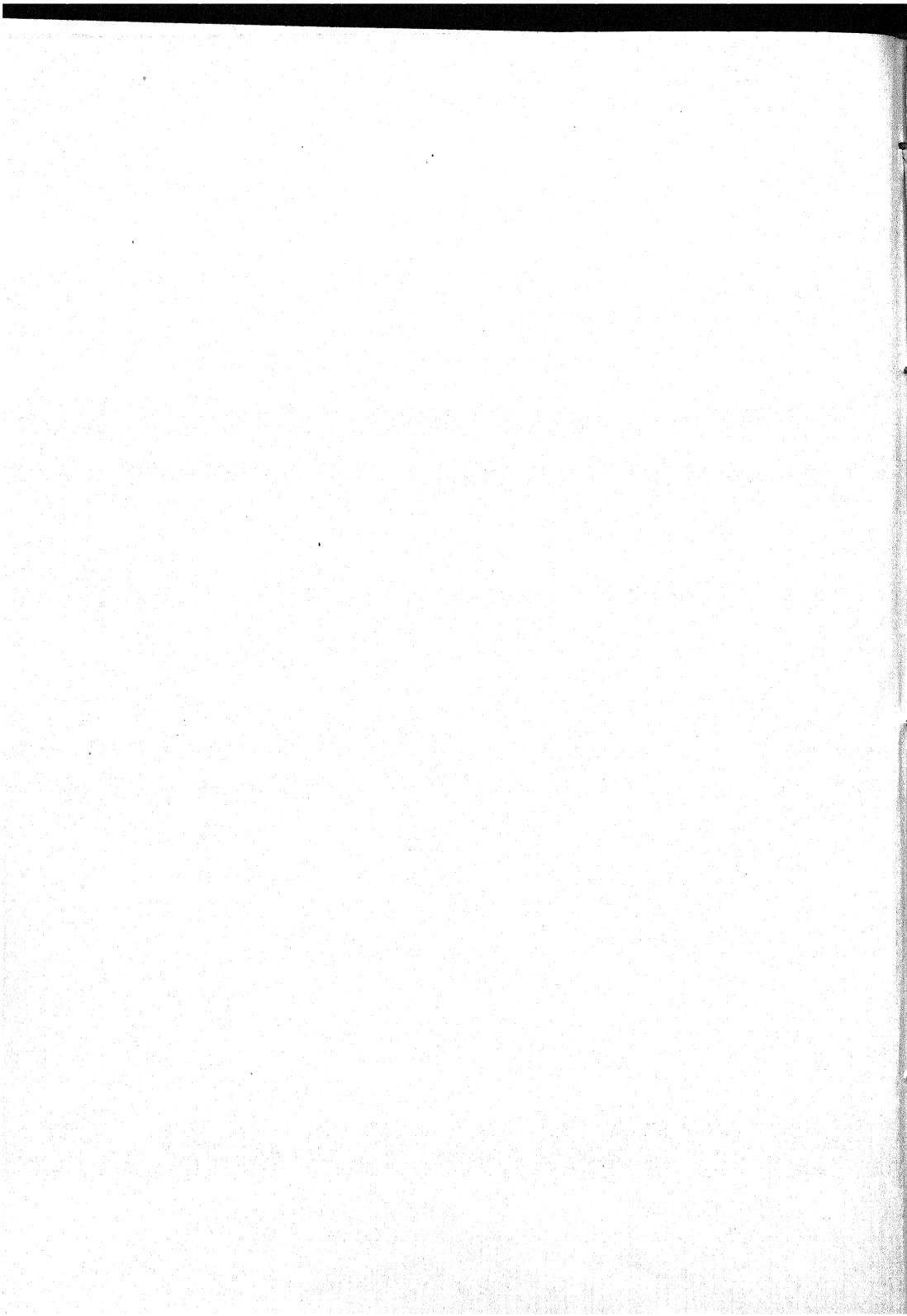
FIG. 32.—Degeneration of 8-nucleate embryo sac.  $\times 800$ .



REES-LEONARD on SOLANUM







## THE MONIMIACEAE AND A NEW LAURELIA

EDWARD W. BERRY

(WITH TWO FIGURES)

The family Monimiaceae does not reach the North Temperate zone, consequently it is unfamiliar to students of fossil floras who have largely lived in that zone. It is a family of great interest to students of plant distribution. Small in size, it contains between 25 and 30 genera and between 175 and 200 species. Systematists have usually recognized a relationship with the Lauraceae and now both families are included in the order Laurales. With the exception of *Laurelia*, no genus is common to both the Old and the New Worlds. Three genera are confined to New Caledonia and only *Tambourissa* of the Old World genera is found in more than one continental area. Nearly half of the known genera are monotypic in the existing flora. But two genera have more than a few species, and both of these, *Mollinedia* and *Siparuna*, with more than 100 species between them, are confined to America. The pertinent facts of the modern distribution are summarized on the accompanying map (fig. 1), to which the reader is referred in lieu of a lengthy discussion.

Only the following six genera have been recognized in the fossil state: *Hedycaria*, *Peumus*, *Mollinedia*, *Monimia*, *Laurelia*, and *Siparuna*, and several of these are of somewhat uncertain status. In addition SAPORTA described three species from the basal Eocene of France for which he proposed the extinct genus *Monimiopsis*.

Four species of *Hedycaria* have been recorded from the Oligocene and Miocene of Europe and a fifth from the Tertiary of Australia. The genus *Mollinedia* has been recorded from the European Miocene and from the Tertiary of Seymour Island. *Monimia* has been recorded from the Oligocene of southern Europe and from Australia. *Siparuna* has been recognized in a warm climate Eocene flora from Oregon and it seems likely that it may be represented in the Eocene floras of southeastern North America.

Six fossil species have been referred to *Laurelia*, and these are



referred to in connection with the following description of a new species from northern Patagonia. The latter comes from a locality on the Rio Pichileufu in northern Rio Negro Territory, about 30 miles east of Lago Nahuel Huapi (latitude  $41^{\circ} 10'$  south, longitude  $70^{\circ} 52'$  west).

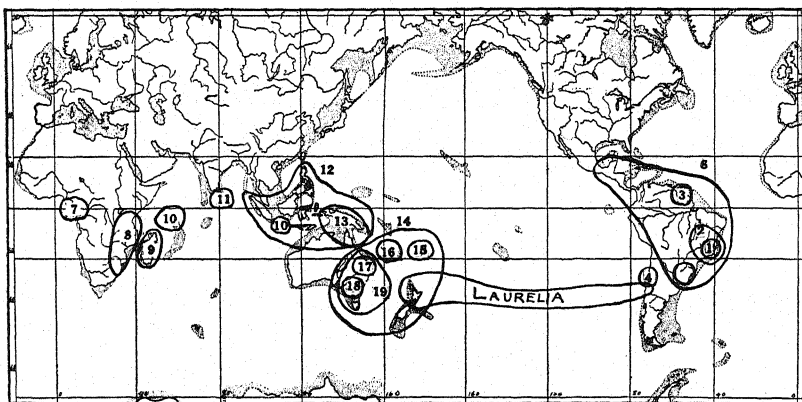


FIG. 1.—Map showing geographical distribution of genera of Monimiaceae: 1, *Macrotorus*; 2, *Macropeplus*; 3, *Conuleum*; 4, *Peumus*; 5, *Hennecartia*; 6, *Siparuna* and *Mollinedia*; 7, *Glossocalyx*; 8, *Xymalos*; 9, *Ephippiandra*; 10, *Monimia* and *Tambourissa* in Mascarenes, *Tambourissa* in Java; 11, *Hortonia*; 12, *Matthaea* and *Kibara*; 13, *Levieria* and *Palmeria*; 14, *Hedycarya*; 15, *Trimenia*; 16, *Amborella*, *Carnegiea*, and *Nemuaron*; 17, *Piptocalyx* and *Daphnandra*; 18, *Doryphora*; 19, *Antherosperma*.

### *Laurelia guinazui* Berry sp. nov.

Leaves of variable size, broadly lanceolate to oval in outline with cuneate base and pointed apex, usually widest near the middle and narrowed about equally proximad and distad. Margins entire at base, elsewhere with prominent serrate teeth, which are conspicuously glandular tipped. Texture subcoriaceous. Length ranging from 5 to 12 cm., normally 7.5 to 11 cm. Maximum width ranging from 2.4 to 6 cm., normally 3 to 3.5 cm. Petiole relatively very stout, usually curved, slightly over 1 cm. in length. Mid-vein stout, not especially prominent. Secondaries six or seven ascending, regularly curved camptodrome pairs, giving off prominent tertiary branches to the marginal teeth. Tertiaries forming an open mesh. Areolation fine, polygonal, isodiametric (fig. 2).

These leaves have the general form and toothed margin similar to that found in a variety of unrelated genera of Flacourtiaceae, Sapindaceae, Proteaceae, and various other families but differ in

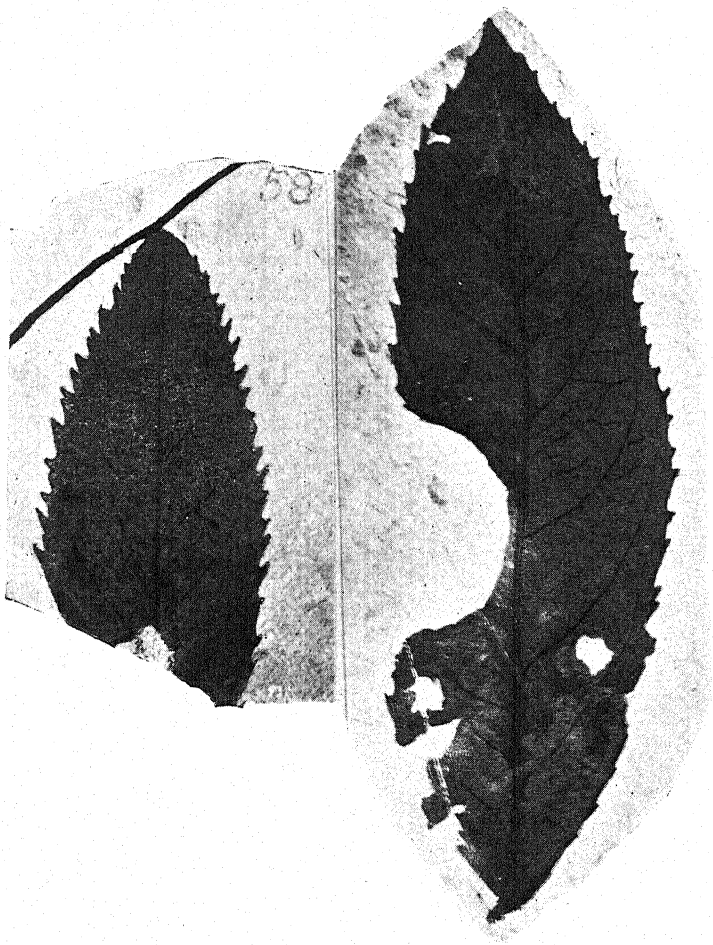


FIG. 2.—Limits of variation in *Laurelia guinazui* Berry, sp. nov.

having glandular marginal teeth. It is this last feature added to the general similarity of the other features to the leaves of the existing species of *Laurelia* that confirms the identification.

The genus is a small one in the existing flora found in Chile and

the northern part of New Zealand. The two Chilean species, *L. sempervirens* and *L. serrata*, occur near the western Andean border of the territories of Rio Negro and Chubut, not far from the fossil locality, in the former approaching to within perhaps 30 or 35 miles of the latter.

The geological record is very incomplete and inconclusive because of the difficulty of recognizing the leaves, but the present day distribution of the surviving species is conclusive enough evidence that an extended geological history awaits discovery. LESQUEREUX many years ago described a species from the Dakota sandstone of Kansas, but this can hardly be accepted as conclusive evidence of the existence of the genus in the mid-Cretaceous of North America. Similarly ENGELHARDT described a species from the Upper Eocene of Germany which is equally doubtful. A European Miocene species described from Croatia by UNGER is probably a member of the family Ternstroemiaceae. ETTINGSHAUSEN described a species from the Lower Miocene of Kutschlin, Bohemia, which looks very like *Laurelia*, but about which no certainty can be expressed. DUSÉN<sup>1</sup> has described a species, considered to be of Oligocene age, from Seymour Island on the border of Antarctica, and a second Argentine species was described<sup>2</sup> a few years ago from Santa Cruz territory.

The present species differs from the last of these, *Laurelia amarillana* Berry, in its average size being somewhat larger, in its slightly more oblong shape, in its more curved and wider initial divergence of the secondaries, in its more regular teeth, and in its more conspicuous glands. In all of these features it departs in the same way from the existing *Laurelia* of South America, *Laurelia amarillana* being practically identical with the latter.

Both of these fossil South American species as well as that from Seymour Island are much more like the existing South American species than they are like the existing New Zealand species. The last has leaves which are smaller and rounder and with more typically crenate marginal teeth.

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<sup>1</sup> DUSÉN, P., Schwed. Södopolar-Exped. 3: p. 4, pl. I, fig. 5. 1908.

<sup>2</sup> BERRY, E. W., U.S. Natl. Mus. Proc. 73: p. 21, pl. V, fig. 3. 1928.

## THE LIGHT LINE IN MELILOTUS ALBA

DOUGLAS H. HAMLY

(WITH FOUR FIGURES)

### Introduction

In a previous article on the seeds of *Melilotus alba*, the writer (3) disagreed with the opinions of former investigators concerning the nature of the "light line" which crosses the Malpighian cells just within the caps. At that time it was not possible definitely to demonstrate the cause of its appearance; hence the present paper gives the results of further study of these light lines.

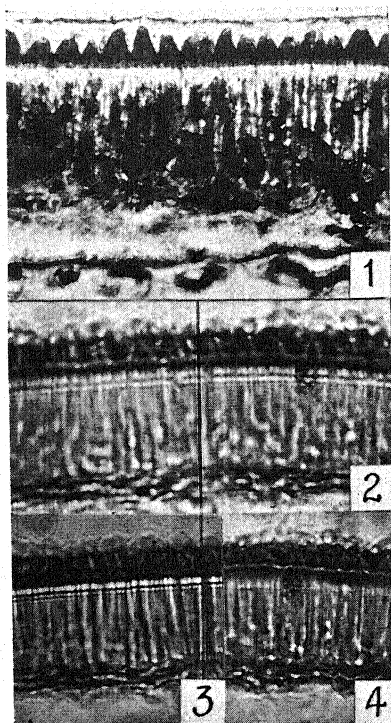
### Investigation

Under ordinary conditions of observation with diffused light, the light line in *Melilotus* appears as a single, rather broad band. It crosses the cells at the interface between the suberin caps and the cellulose bases as described in the paper just cited. This interface is easily seen under the usual conditions of observation. Irregularity may occur, however, as may be observed in a series of photographs made with ultraviolet light by Dr. A. KÖHLER of Jena. Since suberin is much more opaque than cellulose to this light, the caps appear very dark (fig. 1). In the original photographs the configuration of the interface, the finger-like processes of cellulose around the lumen, and the fringed margin of the suberin caps are clearly visible.

With carefully adjusted non-polarized illumination the light band may be resolved into two separate lines (fig. 2), one line occurring within the cellulose and the other within the suberin. The independence of these lines is demonstrated as the objective of the microscope is raised above the plane of best focus.

Thus analyzed, the broad and somewhat hazy light line is seen as two lines, each behaving as a BECKE (1) line such as is found at the surfaces of adjoining transparent substances which differ in refractive index. The BECKE line always moves toward the substance of higher index as the tube of the microscope is raised.

The evidence that the light line is in reality two associated BECKE lines is confirmed by examining the material in polarized light (figs.



FIGS. 1-4.—Fig. 1, photograph of Malpighian cells taken by ultraviolet light with Cd  $0.257\mu$  line, N.A. 0.15.  $\times 508$  (KÖHLER). Figs. 2-4, photographs of same  $15\mu$  section from the same plane slightly above plane of best focus. (KÖHLER illumination with ribbon filament lamp as the source. Wratten M filters C and H used in combination.)  $\times 455$ : fig. 2, in non-polarized light; fig. 3, in light polarized parallel to axes of Malpighian cells; fig. 4, in light polarized perpendicular to axes of Malpighian cells.

3, 4). Figure 3 is a photograph made with light polarized in a plane parallel to the axis of the Malpighian cell. Under these conditions the faint line within the suberin caps is absent, and only the brighter line on the cellulose side remains. The light in figure 4 is polarized in a plane at right angles to the axis of the Malpighian cell. In this case the line is visible in the suberin while that in the cellulose has disappeared. For figures 2, 3, and 4, the lighting was arranged according to the method of KÖHLER (4). To emphasize the separation of the lines the photographs were made from sections placed slightly below the plane of sharp focus.

The immersion methods of CHAMOT and MASON (2) were used to determine the refractive indices of the Malpighian cell constituents. The data for the refractive indices given in table I differ slightly from those reported for pure cutin and pure cellulose.

With light vibrating parallel to the axis of the Malpighian

cells the index of the cellulose base is greater than that of the suberin cap by 0.031, a difference great enough to produce a bright BECKE line which moves toward the cellulose as the tube of the microscope

is raised. When the plane of polarization is perpendicular to the axis of the cell the index of the suberin exceeds that of the cellulose by 0.019. This produces a faint BECKE line on the suberin side of the surface of contact. It is thus definitely indicated that the conditions produced by the juxtaposition of the cellulose and the suberin are such as must give rise to the phenomenon known as the light line.

TABLE I  
REFRACTIVE INDICES OF MALPIGHIAN CELL CONSTITUENTS

MATERIAL	POLARIZATION OF LIGHT	
	PARALLEL TO AXES OF MALPIGHIAN CELLS	PERPENDICULAR TO AXES OF MALPIGHIAN CELLS
Suberin caps.....	$1.554 \pm 0.0005$	$1.556 \pm 0.0005$
Cellulose walls below the caps.....	$1.585 \pm 0.003$ ( $n_a$ )	$1.537 \pm 0.003$ ( $n_g$ )
Difference in refractive index.....	0.031	0.019

The writer gratefully acknowledges the assistance of Dr. A. KÖHLER, and the advice and criticism of Professor H. B. SIFTON, of the Department of Botany and Professor A. L. PARSONS, of the Department of Mineralogy and Petrography, of the University of Toronto.

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## SEED AND SEEDLING OF ACORUS CALAMUS

MURRAY F. BUELL

(WITH THREE FIGURES)

*Acorus calamus* L. grows in eastern and tropical Asia, in Europe, and in eastern North America. In Europe, where it has been introduced and naturalized within historic times, it appears to be uniformly sterile. This is also true of much of the material growing in the older-settled parts of the United States, material which was probably introduced from Europe. In the northern interior, however, where it has every appearance of being native, it fruits abundantly.

It would seem that a study of the seed and seedling of this plant has been neglected in this country, and European observations apparently have been perforce limited to the earliest stages of the ovules. Both MÜCKE (4) and JÜSSEN (3), investigating the causes of the sterility of the European plant, found early degeneration of the embryo sac and very few normal pollen grains. MÜCKE also made some observations on ripe seeds of *Acorus calamus* obtained from Asia, but apparently his supply of these was very limited. He made rather full observations on the development of the seeds of *A. gramineus* Soland, a second Asiatic species. The following observations are submitted at this time; more extended studies on the embryogeny are under way.

### Observations

#### FRUIT AND SEED

The fruit is a several-seeded (1-9, most frequently 5-7), three-locular, dry berry. It is 4-5 mm. long, roughly wedge-shaped, narrowing toward the base, and somewhat 4-angled as a result of crowding on the spadix. The scarious pericarp is lustrous, light brown to straw-colored, and marked with longitudinal dark brown streaks. The septa are thin and delicate. The seeds are borne in a cluster on an axile placenta near the summit of each locule. Although the ma-

ture locules are practically filled with the closely packed, crowded seeds, they contain in the interstices a clear, transparent mucilage, hard and brittle in the mature dry fruit but rapidly absorbing water and swelling into a soft gelatinous mass when dampened. Each seed is invested by a somewhat denser sheath of the mucilage. This mucilage is derived earlier in the development from glandular hairs at the base of the ovules. As a rule all the ovaries on the spadix develop seeds, although occasionally there is some abortion.

MÜCKE'S (4) brief description of the fruit of Asiatic *Acorus calamus* is essentially in agreement with this, except that he found fewer

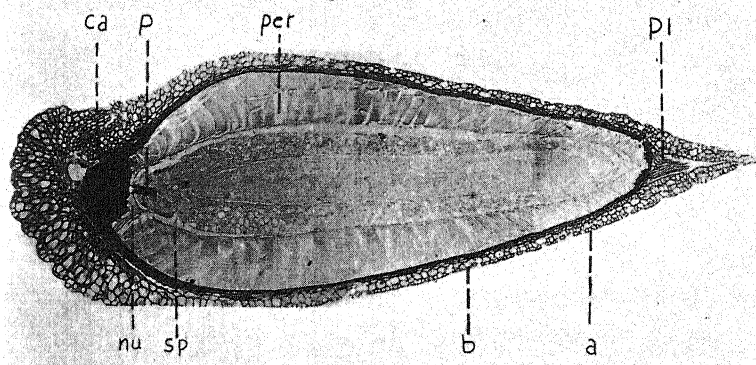


FIG. 1.—Longitudinal section of seed of *Acorus calamus*.  $\times 31$

seeds (2-5) in each fruit. EATON (2) likewise described *Acorus* fruit as fewer seeded. Both BRITTON (1) and SMALL (5) describe *Acorus* fruit as "2-3-celled," but in the present material it is regularly 3-celled; empty locules of semi-abortive fruits may readily be overlooked in the dry condition.

The orthotropous seeds are 3-4 mm. long, narrowly ovoid, and somewhat angled as a result of crowding. The chalaza tapers into the short funiculus which may be either straight or curved or nearly absent, depending upon the position of the individual seed in the locule. The outer surface is light brown, speckled with dark brown indentations. Under the microscope it glistens owing to the air filled parenchyma cells that make up the tissue of the testa. This is 3-4 cells thick, soft, spongy, and easily scraped away. When cut



or bruised it is strongly aromatic. At the micropyle it extends out beyond the nucellus in finger-like lobes.

The tegmen, derived from the inner integument, is thinner and is composed of two layers of cells. These are narrow, elongated, with pointed ends. They fit together tightly and each cell is reinforced with spiral thickenings. The result is a firm, tough, dark brown jacket. At the chalaza this tissue is interrupted by a weak cap of dead cells (fig. 1 *ca*). These are small, parenchymatous, and compactly arranged. In sections stained with safranin or haematoxylin they take an intense stain. In the micropylar region the cells of the inner integument are elongated, coiled about one another, and form a plug in the lower part of the micropyle (fig. 1 *pl*). This inner seed coat everywhere fits tightly against the perisperm. Even at the apex, where the surface of the latter is runcinate, its irregularities are filled in with a firm "cement" formed from disintegrated cells of the tegmen.

The inner cells of the nucellus become entirely disintegrated except for a few remnants which may be seen between the perisperm and the endosperm, while its outer layer persists in the ripe seed as a tough, callous, transparent perisperm one-fifth to one-quarter the thickness of the entire seed (fig. 1 *per*). It is a single layer of cells<sup>1</sup> forming a thick, continuous jacket about the endosperm, except at the base where it is interrupted by the cap of degenerate cells already mentioned. Its cells have the form of truncated wedges radially arranged about the embryo sac. Especially toward the chalazal end of the seed they are somewhat curved, with the concave side toward the chalaza. The walls between these cells are very thin. The cell contents are glassy-clear and homogeneous, no structure whatsoever being observable in fresh material even under the high power of the microscope.<sup>2</sup> MÜCKE (4), working with the corresponding tissue in *A. gramineus*, found it to be protein in composition, and states that it is absorbed at a very early stage of germination. In *A. cala-*

<sup>1</sup> The appearance of more than one layer seen on the upper side of figure 1 is due to the slight obliquity of the plane of the section.

<sup>2</sup> The granular appearance of the cell contents seen in figure 1 results from their having been treated with hydrofluoric acid. Without this procedure it is impossible to obtain microtome sections of the perisperm.

*mus* this process seems to be somewhat delayed, but during germination the perisperm soon softens, and in the later stages it disappears entirely even while the tip of the cotyledon is still surrounded by a layer of unabsorbed endosperm.

The soft endosperm is made up of thin walled parenchyma cells filled with abundant oil and protein food material. It surrounds the embryo completely. A thin layer of crushed cells belonging to the inner part of the nucellus separates it from the perisperm, and a few less crushed cells of this tissue remain about the base of the embryo sac (fig. 1 *nu*). The endosperm is homogeneous throughout except that it is a little more densely filled with stored food in the basal region. Not far from the basal end there is evident in some cases the remnant of a transverse septum which seems to indicate an early division of the embryo sac into two parts (fig. 1 *sp*).

Conspicuous in the longitudinal section of the seed is a structure similar to that which WESTERMAIER (6) has described (in the genus *Aconitum*) as a pedestal (Postament). This appears in the ripe seed as a hard knob of degenerate cells which stands out sharply in stained sections (fig. 1 *p*). It is the remains of a non-vascular conducting strand, which earlier served to supply the embryo sac, together with some hardened remnants of adjacent cells. The downward enlargement of the embryo sac takes place at the expense of the abundant nucellar tissue that originally surrounds this strand, but the process of disintegration has but little effect on the more resistant conducting strand itself.

The cylindrical embryo lies in the axis of the endosperm. It is about 1.5 mm. long. To the base there is still attached a small suspensor (fig. 2 *A, s*). The base of the cotyledon sheathes the plumule (fig. 2 *B*) while its terete limb extends far beyond, forming about three-quarters of the length of the entire embryo.

#### SEEDLING

Seeds collected in September and planted in the University greenhouse germinated readily the following spring. Freezing temperatures during the winter tended to shorten the rest period. Those seeds which have undergone the normal rest period or have been subjected to freezing temperatures show signs of germinating within about

10 days. The radicle is forced through the micropyle as the cotyledon begins to elongate. Almost immediately the radicle turns downward so that the rapidly developing primary root is directly established in the soil (fig. 3 *A*, *B*). Simultaneously with commencement

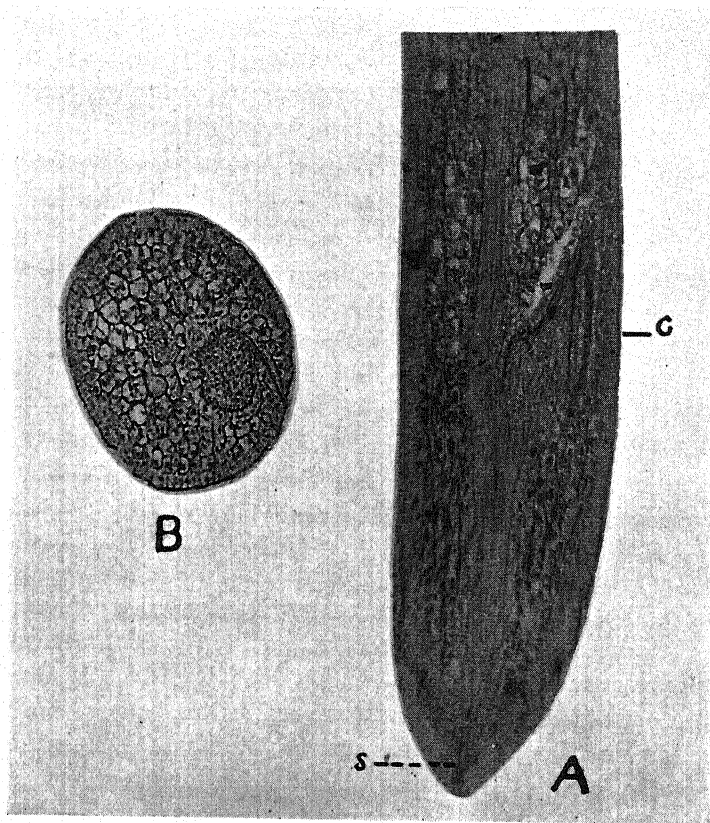


FIG. 2.—*A*, longitudinal section of embryo between *a* and *b* of fig. 1; *B*, cross section of embryo at *c* in adjacent figure.  $\times 150$ .

of root growth the hypocotyl begins to enlarge slightly (fig. 3 *A*, *sw*), and at this swelling appears a band of hairs, which doubtless serve as auxiliary organs to absorb water and minerals while the primary root is yet in its embryonic stages (fig. 3 *B*, *C*, *D*, *h*). These primary absorbing organs, together with the immediate production of chloro-

phyll in the emerging embryo, lead to the early independence of the plant.

While the primary root develops rapidly, it remains small and is soon replaced by numerous adventitious roots. The cotyledon con-

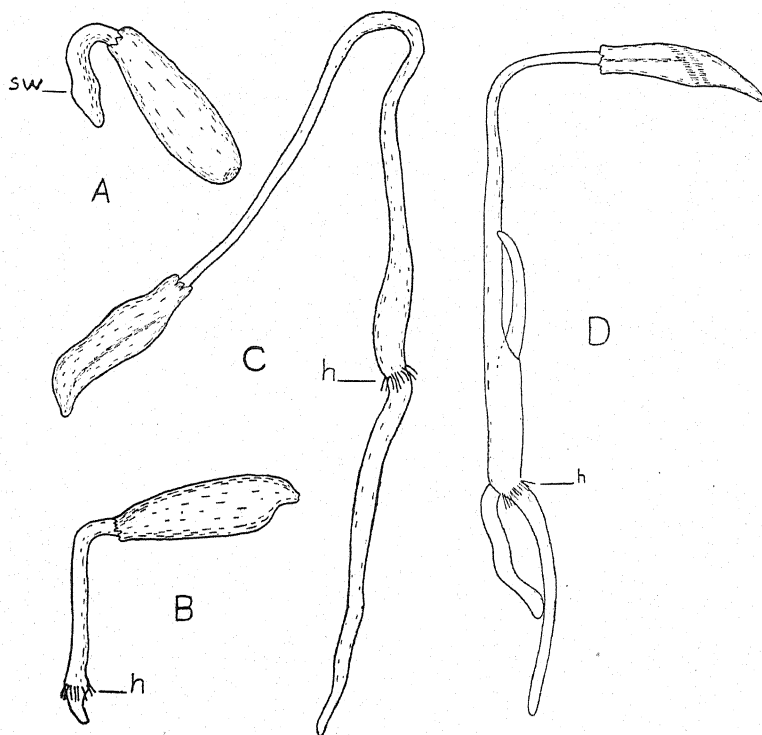


FIG. 3.—Stages in development of the seedling.  $\times 4.2$

tinues elongating, lifting the remains of the seed into the air, while its tip remains in the endosperm, absorbing the last of the stored food. At maturity of the seedling, that is, as the first plumular leaf is about to emerge, the cotyledon attains a length of about 20 mm., its sheathing base forming one-fifth to one-quarter of its whole length. There is no ligule on the cotyledon as there commonly is in monocotyledonous seedlings. The first plumular leaf appears about simultaneously with the first adventitious root (fig. 3 D).

In the dormant embryo the vascular system is indicated by a pro-

cambial strand which can be traced from the apex downward along the axis of the cylindrical cotyledon and into the hypocotyl region (fig. 2 A). Slightly above the base of the plumule it becomes more distinct. In the hypocotyl it is best developed on the side directly below the median plane of the cotyledon and somewhat less sharply differentiated on the side that lies below the plumule. The plumule itself is as yet completely undifferentiated meristematic tissue. The latter, however, develops rapidly during germination; and about simultaneously with the first conspicuous indications of conducting tissue in the rudimentary primary root, the midrib of the first plumular leaf becomes evident and at the same time there appears the first differentiated xylem in the base of the cotyledon.

In the mature seedling the vascular tissues of the cotyledon and primary root are well differentiated, and the three main traces of the first plumular leaf and a single stem bundle are readily discernible. In the limb of the cotyledon there is a single vascular strand with collateral xylem and phloem. As this passes downward through the brief transition region the exarch condition is attained and the root structure rapidly appears. The root is diarch, one pole developing directly beneath the cotyledonary strand and the other directly in line with the midrib of the first plumular leaf. The endodermis, present in the root and the rhizome of the plant, is already differentiated in the seedling at the first node.

### Summary

1. *Acorus calamus* L., which fails to produce seed in Europe, fruits freely in Minnesota, where it is probably native.

2. The fruit is a 3-celled, gelatinous, dry berry containing usually 5-7 orthotropous, ovoid, somewhat angled seeds pendent from an axillary placenta. The cylindrical embryo lies in the axis of the endosperm, which is surrounded by a thick callous perisperm. The seed coat is made up of a thin tough tegmen surrounded by a thicker spongy testa.

3. As the seed germinates the tip of the cotyledon remains in the seed as an haustorial organ and lifts it into the air as growth continues. The seedling immediately becomes green and develops absorbing hairs so that it is early independent. The single vascular

bundle of the cotyledon passes directly down into one pole of the diarch root while the midrib of the first plumular leaf is directly continuous with the other pole.

I wish to thank Dr. C. O. ROSENDAHL and Dr. F. K. BUTTERS who suggested the problem and gave valuable assistance during the course of the work.

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## CURRENT LITERATURE

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### Genetics of garden plants

According to the preface, the aim of this book<sup>1</sup> is twofold: "First, to give an introduction to the essential principles of genetics and cytology; and secondly, to give an account of recent results in relation to horticulture." The first objective as it relates to genetics seems to be doubtfully attained, while the second is admirably handled.

The general principles of genetics are condensed into one short chapter of 12 pages. Even for the lay horticulturist, this brevity means unbalance and serious omission. Nowhere is the theory of quantitative inheritance discussed; the principles of heterosis are granted one short sentence; and most surprisingly, the whole question of inbreeding is totally ignored. Obviously no one can do justice to a field like genetics in so brief a treatment, but it may serve to spur the reader to further study.

Cytological phases are clearly and concisely developed; particularly the chapter on the cytology and genetics of polyploids is very well reviewed.

There is a noticeable tendency to minimize and overlook much of the better continental European and American research on genetics. For example, the treatment of *Dahlia* is exhaustive while that on *Antirrhinum* is almost neglected. The German, Russian, and American work on *Cucurbita*, *Lycopersicum*, *Phaseolus*, and many other garden crops is either ignored or underemphasized. The bibliography is correspondingly incomplete and unbalanced. The history and genetics of the sweet pea, the garden stock, *Primula*, the garden pea, and the potato are exceptionally well treated.

A major share of the book naturally deals with the special interests of the authors. Here the discussions on fruit breeding, genetics, and cytology are extremely well presented, as is the recent work on sterility and incompatibility. The advanced student of horticulture will certainly need this book for his field of research, and will find the material clearly and convincingly written. In fact, did the title read "Recent advances in cytology and breeding of garden plants" instead of its actual, very broad title, many of the preceding criticisms would be unnecessary. In general the format is excellent and the illustrations original and instructive.—E. W. LINDSTROM.

### Physico-chemical properties of plant saps

The untimely death of Dr. J. ARTHUR HARRIS left a great mass of uncompiled and unpublished data on the physico-chemical properties of plant saps which he

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<sup>1</sup> CRANE, M. B., and LAWRENCE, W. J. C., The genetics of garden plants. 8vo. pp. xvi+236. figs. 53. Macmillan, London. 1934.

collected during many seasons' field work in both the eastern and western United States, the Hawaiian Islands, and Jamaica. Although very regrettable that Dr. HARRIS could not have been spared to complete this work himself, it is extremely fortunate that these data, consisting of approximately 12,000 series of determinations of freezing point depression, specific electrical conductivity, chloride and sulphate content in grams per liter of sap, and occasional determinations of hydrogen ion concentration, have been made available in book form for future workers.<sup>2</sup> The University of Minnesota, particularly President COFFMAN, the editorial committee consisting of Dr. C. O. ROSENDAHL, Dr. GEORGE O. BURR, and Dr. ROSS AIKEN GORTNER, and all who participated in the project are to be complimented upon bringing this most worthy project to fruition.

The volume contains sufficient material for several other monographs or articles if the data were thoroughly analyzed, studied, and correlated. Part I consists of short papers and memoranda on the physico-chemical properties of plant saps in relation to plant geography. These are by Dr. HARRIS, with the exception of a short paper by Dr. H. L. SHANTZ on the importance of phytochemical studies in the field of plant geography.

Part II contains the experimental data systematically arranged by botanical names of species under each state. An easily understood code and symbols are used to facilitate publication in as brief form as possible. A separate index to the data has been appended for use by those wishing to make ecological studies of the plants in particular communities. This index will probably be used extensively in conjunction with the descriptions of stations.—C. F. KORSTIAN.

#### Reproduction in fungi

Following rather closely the appearance of the fifth volume, the sixth volume of BULLER's monumental work<sup>3</sup> is, in effect, a continuation of the second part of its predecessor, dealing as it does with reproductive mechanisms in various fungi, most of which involve violent discharge.

The present volume is divided into three parts, the first (and longest) being concerned with the genus *Pilobolus*. There is the usual complete survey of earlier literature, followed by a detailed account of the development of the *Pilobolus* sporangium and its discharge. Numerous illustrations show the manner in which the subsporangial swelling is acted upon by the incident rays of light so as to aim and discharge the sporangium in the direction of the light source. *Ocellus*, the term first suggested by BULLER in 1921 for the subspo-

<sup>2</sup> HARRIS, J. ARTHUR, The physico-chemical properties of plant saps in relation to phytogeography. Completed and edited by C. O. ROSENDAHL, G. O. BURR, and R. A. GORTNER. Univ. Minnesota Press. pp. vi+339. 1934.

<sup>3</sup> BULLER, A. H. R., Researches on fungi. Vol. VI. pp. xii+513. figs. 231. Longmans, Green and Co., London. 1934.



rangial swelling, is chosen with evident intention to emphasize the eye-like character of the structure.

Taxonomists will welcome the systematic treatment of *Pilobolus* and *Pilaira*, contributed by W. B. GROVE, which constitutes the fourth chapter. On p. 190 the Pilobolidae is referred to as a "section of the Mucorini," while on p. 200 it is said to be a "family of the Mucoraceae." Presumably the author regards the group as a family, but if so, it is to be regretted that he has not seen fit to follow accepted modern usage and write Pilobolaceae.

The second part deals with the phenomenon of puffing in the Discomycetes. Working with representatives of several genera, and on the basis of experiment as well as observation, the author concludes that the simultaneous discharge of a large number of asci is of definite advantage to the fungus, in that it produces a blast of air capable of carrying the spores for several inches after they have lost their original velocity. The direction of discharge is governed in part by the position of the operculum and in part by heliotropic response of the ascus tip, the details varying in different species, but the aim always so adjusted as to secure a parallel direction of discharge for all asci in a single ascocarp, whatever their position in the hymenium.

The third part deals with pseudorhizae and gemmifers as organs of Hymenomycetes. BULLER adopts FAYOD's term "pseudorhiza" as more accurate than the term "rooting base" used to designate the subterranean part of the stem of the basidiocarp of a number of fungi, and perhaps best illustrated in the familiar *Collybia radicata*. Study of the development of the pseudorhiza in connection with the subaerial stipe in *Coprinus macrorrhizus* makes it clear that the pseudorhiza is merely the lower portion of the stipe, serving as a connecting link between a mycelium immersed in a buried matrix and the subaerial part of the fructification. Of particular interest is the perennial, branching pseudorhiza of *Collybia fusipes*.

The final chapter has to do with the so-called "Stilbum" heads of the coffee leaf-spot fungus, *Omphalia flavida*. It is shown that these are in reality sterile, modified basidiocarps, in which the entire pileus is detached and serves as a disseminule. Observations on the luminosity of the species are recorded and comparison is made with the reproductive bodies of *Sclerotium coffeicola*. STAHEL's view that these may represent sterile, modified basidiocarps of a *Typhula* (essentially similar in nature to the gemmifers of the *Omphalia*) is quoted with approval.

As in the previous volumes, the numerous well selected and fully explained figures and the excellent general summary and index greatly facilitate the use of the volume.—G. W. MARTIN.

#### A new mycological text

There has long been need for a comprehensive treatment of the fungi in English, suitable for use with classes whose members have had no introduction

to the subject other than that received in elementary courses. Teaching mycologists will welcome, therefore, the appearance of BESSEY'S new book.<sup>4</sup>

After an introductory chapter, which includes a short history of mycology, a chapter is devoted to forms commonly included in the fungi, but which the author regards either as unrelated or at most doubtfully related to the true members of the group. Such forms include the slime molds, Acrasiales, Labyrinthulales, Plasmodiophorales, and Chytridiales. The true fungi are held to begin with the filamentous Phycomycetes, whose derivation from green algae is favored, while the higher fungi are regarded as constituting a distinct phylum, the Carpomycetae, traceable to the red algae. Quite properly, these views on phylogeny determine the order of presentation.

The book is well balanced. The Phycomycetes are adequately covered in three chapters (67 pages), with frequent reference to FITZPATRICK'S work, which, however, is by no means slavishly followed. The Carpomycetae are divided into three classes, the Ascomycetae, Teliosporae, and Basidiomycetae. The feminine plural, instead of the more familiar masculine ending, is adopted for all groups but the Fungi Imperfecti, presumably to emphasize the author's frequent allusion to the fungi as plants. The treatment of the Ascomycetes (127 pages) begins with the Laboulbeniales, Lecanorales, and Pezizales as showing most resemblance to the postulated red algal ancestral forms, and ends with the Aspergillales and Saccharomycetales, this sequence being adopted in accordance with a concept of progressive reduction. The introduction of the Lecanorales and Pyrenulales into the series is a welcome approach toward a more rational handling of the lichens.

The recognition of the Teliosporae, including the rusts and smuts, as a class coordinate with the other two groups of higher fungi (43 pages) is unlikely to find general acceptance. In accordance with this view, however, the terms basidium, basidiospore, and pycnium are regarded as inapplicable, the older terms promycelium, sporidium, and spermogonium being preferred. The pycniospores are called sperms. The author prefers to derive the Teliosporae from some primitive member of the Pezizales rather than directly from the red algae.

The Basidiomycetes, as restricted, occupy three chapters (60 pages). The Heterobasidia are recognized in the page headings, but not the Homobasidia. The treatment is on the whole conservative and in accord with established usage, with adequate recognition of its tentative character. A brief discussion of the imperfect fungi concludes the text.

To each chapter is appended an admirably selected bibliography, while a guide to the literature for identification of the fungi occupies no less than 76 pages at the end. The method of citing references by groups entails a certain

<sup>4</sup> BESSEY, E. A., A textbook of mycology. pp. xv+495. P. Blakiston's Son & Co., Philadelphia. 1935. \$4.

amount of repetition, but this is probably justified by the resulting convenience and accessibility of the titles.

Typographical errors are not unduly numerous, but the addition of commas in a number of appropriate places would clarify the meaning and occasionally save the sentence, as printed, from approaching absurdity. The author properly argues that smut spores are not chlamydospores (p. 252) but permits himself (p. 95) to refer to the "sterigmata" of *Bremia*. The indiscriminate use of the terms "sexual" for any reproductive process involving or implying nuclear change, and "asexual" for those not involving such change, has been adequately criticized by LINK. It is to be regretted that the latter's proposed substitutes, caryallagic and acaryallagic, have not yet found their way into general use. A somewhat analogous criticism might be made of the frequent and often meaningless adjective "normal." The illustrations are abundant and for the most part helpful, although many of the drawings appear unnecessarily crude, and a few of the photographs, figs. 39 and 107 for example, would convey little to one who was not already familiar with the fungi they illustrate.

A satisfactory textbook in a specialized field, if it is to be of wide usefulness, must necessarily take a conservative position with reference to many debatable points, at the same time making it clear that there are points of view to be considered other than those adopted. This book conforms to such a criterion. The author's judicial attitude, his manifest fairness, the fullness with which he cites opposing views, and his constant suggestion of challenging problems combine to make this a useful and stimulating text.—G. W. MARTIN.

#### Plant life

The list of elementary texts continues to grow. The present work<sup>5</sup> is appreciably shorter and less encyclopedic than some, being expressly designed for a course extending through a single semester.

On the whole, the book is well illustrated; some of the illustrations are refreshingly new and apt while many others are drawn from various popular texts and other sources. Although the author has deliberately centered his method of presentation around processes, structure is not neglected. In many instances, treatment of some of the subjects is abbreviated to little more than a mention of their existence or suspected importance. The usual sequence of forms purporting to represent evolution in plants is given.

One is still left to hope that eventually an introduction to the science of botany may be written and offered as a textbook in the subject. Perhaps the two things are mutually antagonistic. The hope may indeed be vain so long as instructors continue to demand longer and longer abbreviated encyclopedias for use as texts, and the struggle to keep up with this demand grows more and more acute.—E. J. KRAUS.

<sup>5</sup> SWINGLE, D. B., *Plant life*. 8vo. pp. 441. figs. 290. Van Nostrand Co., New York. 1935. \$3.

### Hortus

Those who have been accustomed to turn to Hortus for concise and accurate information on plants of horticultural importance will welcome this new edition,<sup>6</sup> which is brought up to date by the addition of a supplement. Reference to nearly 3000 additional species has been appended, as well as many varieties and form names. The same general style of citation as in previous editions has been followed.—E. J. KRAUS.

### Weeds

Botanists and agriculturists alike will welcome a comprehensive treatise on weeds.<sup>7</sup> The book is divided into two principal parts. Part I affords a history and much general ecological information on weeds, including methods of control. Part II is a systematic listing of a great number of species, together with specific notations of their occurrence and methods of control. An extensive key is provided. Good and clearcut line drawings are used to illustrate many of the species.—E. J. KRAUS.

### General plant cytology

The first volume of the second edition of TISCHLER's work on plant cytology has recently appeared.<sup>8</sup> This volume deals exclusively with the resting nucleus, which is considered under the following headings: resting nucleus and its external morphology; chemical organization; morphological structure of the reticulum and the karyolymph; the nucleolus; protein crystals; relation of the nucleus to plastids, chondriosomes, centrosomes, and blepharoplast; relation of the nucleus to cell wall formation; movement of the nucleus within the cell and its physiological significance; relation of the nucleus to cell division; multinucleate condition of cells; and degeneration and resorption of the resting nuclei. The book is an able and comprehensive summary of the knowledge pertaining to this subject which has been accumulated during the past 75 years and should be a valuable addition to the reference library. The citation of pertinent literature is very complete.—J. M. BEAL.

### A Swiss flora

Two European ecologists have undertaken the task of an elaborate and detailed flora<sup>9</sup> of southeastern Switzerland. This mountainous region possesses an

<sup>6</sup> BAILEY, L. H. and ETHEL Z., Hortus: a concise dictionary of gardening, general horticulture and cultivated plants in North America. New revised edition with supplement. pp. 755. pls. 16; figs. 22. Macmillan Co., New York. 1935. \$5.

<sup>7</sup> MUENSCHER, W. C., Weeds. 8vo. pp. 577. figs. 123. Macmillan Co., New York. 1935. \$6.

<sup>8</sup> TISCHLER, G., Allgemeine Pflanzenkaryologie. Handbuch der Pflanzenanatomie. Band. II. pp. 630. figs. 252. Verlag Borntraeger, Berlin. 1934. RM 64.

<sup>9</sup> BRAUN-BLANQUET, J., and RÜBEL, EDUARD, Flora von Graubünden. Veröff. Geobot. Inst. Rübel in Zürich 7: Erste Lieferung. pp. 1-382. map. 1932; Zweite Lieferung. pp. 385-820. 1933; Dritte Lieferung. pp. 821-1204. 1934. Hans Huber, Bern and Berlin.

annual rainfall ranging from 90 to 150 cm. in the north to 140 to 160 cm. in the south. For the purpose of the flora the montane and lowland portions of the area are divided into a northern, a central, and a southern region dominated respectively by forests of beech, pine, and oak. The subalpine and alpine portions of the area, with similar major regions, are subdivided according to the various mountain systems. Special attention is given to the ecological range and habitat conditions of the various species and citations of altitude are many and detailed.

The nomenclature and arrangement are those of SCHINZ and KELLER in their *Flora der Schweiz* but numerous other authorities have been consulted, as indicated by the extensive bibliography. Many Swiss botanists have assisted in the collection and compilation of the data and many herbaria have been consulted.

The work will set a high standard for systematic botanists and will prove of great value to plant geographers and ecologists interested in the distribution and history of European floras. Students of alpine vegetation will find in it an abundance of ecological data from the home of alpine ecologists.

Three numbers have been issued. The first deals with the pteridophytes, gymnosperms, and monocotyledons; the second includes the lower families of the dicotyledons up to and including the Rosaceae; while the third discusses the intermediate families of the dicotyledons from the Leguminosae to the Solana-ceae. It is expected that the concluding number of the volume will appear very soon.—G. D. FULLER.

#### Struggle for existence

This volume<sup>10</sup> summarizes the opinions and theories of the most prominent contributors to our knowledge of this subject. It also reports a series of experimental tests of the mathematical theories of VOLTERRA, LOTKA, and BAILEY, made under rather carefully controlled laboratory conditions. From these experiments GAUSE is able to criticize constructively the present mathematical descriptions of data and to suggest scores of possible problems. His experimental data are concerned first with the struggle for a common limited amount of energy by various organisms, as for example yeast cells (*Saccharomyces cerevisiae* vs. *Schizosaccharomyces kephir*), and second with prey-predator relations between various species of *Paramecia* and *Didinium*.

The genuinely critical attitude of the author is stimulating, and the mathematical treatments are so simply explained that the book should aid the non-mathematically inclined experimenter to understand the highly technical works of LOTKA, VOLTERRA, and BAILEY.

The tone of the book is distinctly introductory and tentative, treating only a very small portion of the possible fields of investigation, and it is to be hoped that it will be followed by a more complete and comprehensive treatise on the subject.—R. B. OESTING.

<sup>10</sup> GAUSE, G. F., *The struggle for existence*. pp. 163. Williams and Wilkins Co., 1934. \$3.

## Southern California botany

Students and traveling botanists will welcome the appearance of this authoritative and compact treatment<sup>11</sup> of the plants of an area smaller in size than those covered by the larger works of ABRAMS and JEPSON. MUNZ acknowledges the influence of these authors' publications on his own volume, but points out that most of our western states are so poorly known botanically that any student of a local flora can quickly discover new facts. His observations over a period of nearly two decades are summarized in the present volume.

The area covered includes San Diego, Imperial, Los Angeles, Orange, Riverside, San Bernardino, and Ventura counties, portions of Santa Barbara, Kern, and Inyo counties, as well as the Channel Islands. It is thus a region of very diverse floristic, climatic, and topographic relationships. A hasty tabulation by the reviewer shows that approximately 2700 species and 750 genera are found in the area, of which a large number of the former are endemic. At one climatic extreme arctic-alpine elements are found on San Gorgonio and San Jacinto peaks, while at the other Lower Sonoran elements dominate in the Colorado and Mohave deserts.

A number of useful innovations not commonly found in manuals are a discussion of the distribution of southern California plants, a list of nomenclatorial changes, one of persons for whom species have been named, and a list of meanings of specific names, this last prepared by F. W. PEIRSON. The glossary and index seem adequate and accurate. The list of abbreviations of authors' names is very brief.

The descriptive flora covers 596 pages, the arrangement of families following the Englerian system in the main. Descriptions are adequate for the purposes of the book, and the keys seem to be usable. Occasional errors have crept in. The most serious one which caught the eye of the reviewer is in the generic key of the Ranunculaceae. Other errors are more apparently typographical in nature. Three of the author's students prepared the 310 text figures which are included.

Nomenclatorial changes number 98. The majority are new combinations, but two new species, *Phacelia minutiflora* J. Voss and *Monardella robisonii* Epling, as well as eleven new varieties, are described.

Preparation and publication of the book was made possible largely through the generosity of Miss ELLEN SCRIPPS.—C. E. OLMSTED.

## Fossil cycads

A recent paper<sup>12</sup> describes the well known *Cycad* trunk from the Carpathians of Poland which is now found in the Zwinger Museum at Dresden. The description includes the histological features of the woody cylinder, the cortical

<sup>11</sup> MUNZ, P. A., A manual of southern California botany. 8vo. pp. xl+642. figs. 310. Claremont, California. 1935.

<sup>12</sup> WIELAND, G. R., Fossil cycads, with special reference to *Raumeria reichenbachiana* Goepfert sp. of the Zwinger of Dresden. Palaeontographica. 79: ser. B. 85-130. Stuttgart. 1934.

parenchyma, and the flowers. It is suggested that the generic name be changed to *Cycadeoidea*. WIELAND makes comparisons with other *Cycadeoideae* and establishes the great similarity of *Raumeria reichenbachiana* with *Cycadeoidea dakotensis*.—A. C. NOÉ.

#### Low temperature relations of plants

For many years research concerning the low temperature relations of plants has been very active. The problems of freezing injury, the conditions which produce hardiness, the metabolic significance of the hardening processes, varietal differences in resistance to frost injury, inheritance of frost resistance, the significance of unfreezable water, etc., have received wide attention. Correspondingly an extensive literature has developed, widely scattered in journals, many of them obscure, or not obtainable readily in libraries.

An annotated bibliography covering this field of investigation has been prepared by HARVEY<sup>13</sup> and his assistants. The bibliography lists 3412 contributions, arranged alphabetically. A topical index of 28 pages makes it possible to secure the citations to almost any subject one desires. Wherever the titles do not indicate the subject matter of the papers, brief annotations give an idea of their contents.

The bibliography is mimeoprinted, with flexible leatherette binding. It represents a very large expenditure of time and energy. There is need of such bibliographies in the case of extensive and far flung literatures, and future progress will be much more rapid as a result of the unselfish devotion of the author and his aids, who have worked on the compilation for over 15 years. Their efforts will be appreciated by all workers in this field.—C. A. SHULL.

#### Biochemical laboratory methods

MORROW's volume,<sup>14</sup> first published in 1927 shortly after the author's untimely death, has appeared in a second edition, revised and rewritten by W. M. SANDSTROM.<sup>15</sup> The principal changes made concern the order of the experiments, which have now been arranged to conform more closely to the order of presentation in the companion text, *Outlines of Biochemistry*, by R. A. GORTNER. It has been possible to omit some discussion which has been more adequately presented in GORTNER's book. The volume is therefore somewhat reduced in size, 319 instead of 350 pages. It offers 231 laboratory exercises of varying degrees of difficulty. The work has proved very useful, and the new edition will no doubt stimulate much interest in biochemical methods and problems.—C. A. SHULL.

<sup>13</sup> HARVEY, R. B., *An annotated bibliography of the low temperature relations of plants*. 8vo. pp. ii+223. Burgess Publishing Co., Minneapolis. 1935. \$4.

<sup>14</sup> BOT. GAZ. 85:466-467. 1928.

<sup>15</sup> MORROW, C. A., and SANDSTROM, W. M., *Biochemical laboratory methods for students of the biological sciences*. 8vo. pp. xv+319. John Wiley and Sons, New York. 1935. \$3.75.

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